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MLS 532

1. Write extensively on the exfoliative cytology of the female genital tract

Diagnostic cytology is the science of interpretation of cells that are either exfoliated from epithelial surfaces or removed from various tissues. George N Papanicolaou introduced cytology as a tool to detect cancer and pre-cancer in 1928. It is now a widely accepted method for mass screening in asymptomatic population. The advantages of diagnostic cytology are that it is a non-invasive, simple procedure, helps in faster reporting, is relatively inexpensive, has high population acceptance and facilitates cancer screening in the field. Diagnostic cytology can be carried out by different methods, which includes collection and examination of exfoliated cells such as vaginal scrapes, sputum, urine, body fluids etc. Collection of cells by brushing, scraping or abrasive techniques is usually employed to confirm or exclude malignancy. Fiberoptic endoscopes and other procedures can be used for collecting samples directly from the internal organs.

The accuracy of the cytologic examination from anybody site depends greatly on the quality of collection, preparation, staining and interpretation of the material. Inadequacy in any of these steps will adversely affect the quality of diagnostic cytology. Diagnostic accuracy and reliability are major issues in cytology practice.

Methods of specimen collection

Individual cells may be studied in many ways, which include Exfoliative cytology or FNAC/FNAB (Fine-Needle Aspiration Cytology/ Biopsy)

EXFOLIATIVE CYTOLOGY

It is the study of cells that have been shed or removed from the epithelial surface of various organs. Cells from all organs, which communicate with the exterior of the body, are suitable for study. These cells can be recovered either from natural secretions such as urine, sputum and vaginal or prostate fluids or by artificial means such as paracentesis or lavage.

The cells can be collected from the epithelial surfaces by lightly scraping the surface, by swabbing, aspirating or washing the surfaces. Normal cells are cohesive in nature but exfoliated when they attain maturation. During malignant conditions or during infection, the exfoliation becomes exaggerated and the epithelial cells show variation in morphology. Such exfoliated cells, when collected and appropriately stained, give information on the living epithelium from which they are derived. These characteristic cellular and nuclear appearances in cells thrown off from healthy epithelium, differ distinctly from those, derived from inflamed or malignant lesions. Thus, by studying the alterations in morphology of the exfoliated cells and their pattern, the diagnosis of various pathologic conditions can be made.

Female Genital Tract (FGT): The cytological specimens collected from FGT include cervical smear, vaginal smear, aspiration from posterior fornix of vagina (vaginal pool smear) and endometrial smear.

Cervical smear: Cancer of the uterine cervix is the commonest cancer in the FGT. Almost all invasive cancers of the cervix are preceded by a phase of preinvasive disease, which demonstrates microscopically a continuing spectrum of events progressing from cervical intraepithelial neoplasia (CIN) grade I to III including carcinoma in-situ before progressing to squamous cell carcinoma. This progressive course takes about 10 to 20 years. Early detection even at the preinvasive stage is possible by doing cervical smear (Pap Smear Test). This can identify patients who are likely to develop cancer and appropriate interventions may be carried out.

Advantages of Pap Smear:

 ● It is painless and simple

● Does not cause bleeding

● Does not need anesthesia

● Can detect cancer and precancer

● Can identify non-specific and specific inflammations

● Can be carried out as an outpatient procedure

Patient Preparation: The patient should be instructed on the following before coming for smear collection;

* she should not douche the vagina for at least a day before the examination.
* No intravaginal drugs or preparations should be used for at least one week before the examination.
* the patient should abstain from coitus for one day before the examination.
* Smear should not be taken during menstrual bleeding, because of contamination with blood, endometrial component, debris and histiocytes.

Sampling: A cervical cytological sample is considered satisfactory for cytological diagnosis when their composition reflects the mucosal lining of the cervix, encompassing ectocervical, squamous metaplastic cells and endocervical columnar cells in fair numbers. It is generally agreed that majority of epithelial abnormalities that eventually lead to an invasive cancer originate in the squamo-columnar junction (transformation zone). As stated by the British Society for Clinical Cytology (BSCC), a cervical smear if properly taken should contain cells from the whole transformation zone(TZ). The sample should contain a sufficient quantity of epithelial cells, and both metaplastic and columnar cells should be present. According to the Bethesda System, an adequate smear contains an adequate endocervical/transformation zone component. Lubricant should not be used while examining, as it can obscure the cells during smear examination.

Sampling Devices: The collection device may play an important role in sample adequacy. The shape, surface, texture and material of the device may determine how much of the scraped material is deposited on to the glass slide and is available for screening and analysis.

The use of cotton swab for collection of cervical smears is to be discouraged, in view of the drying artifacts and loss of cells, which are caused by this method.

●Ayre’s spatula: Smears obtained with original Ayre’s spatula are often easier to screen. Wooden spatula is preferable to plastic spatula, because of its mildly rough surface that can collect more material. The disadvantages are that the method may occasionally be traumatic to the patient, and the tip of spatula that does not fit the external os (orifice), it may fail to remove some of the valuable material from the squamo-columnar junction.

Based on the original wooden Ayre’s spatula, many devices of different shapes and sizes have been introduced to improve sampling. This includes Endo-cervical Brush, Cervex, Cytobrush, etc.

* The Cervex brush device is a flexible plastic brush, which follows the shape of the endocervix, transformation zone and ectocervix as well and is suitable for every cervix shape.
* Endo-cervical brush is a small bottlebrush like device with one end having fine bristles made up of nylons. This device is strictly for taking materials from endocervix. Gently insert the brush in endocervix and rotate one turn pressing in the upper and lower wall.
* The cytobrush is similar to that of endocervical brush except that the projected tip is without bristles. This can be used for obtaining cells from the whole cervix.

Single sampling devices and methods have their limitations in obtaining adequate smears from the cervix. A combination of two devices, usually spatula and endocervical brush, give better results. Triple smear or the vaginal-cervical-endocervical (VCE) technique can provide the best results. However, feasibility and cost factor need to be taken into consideration. In postmenopausal women, the squamo-columnar junction recedes making it difficult to obtain good amount of endocervical cells and cells from TZ (transformation zone). Hence a combination of two devices, spatula plus endocervical brush is preferred. In those with a prolapsed uterus, the cervix is first soaked with normal saline and scrape is collected with cytobrush. To obtain a satisfactory smear from a bleeding cervix, the blood is wiped with wet cotton and smear is obtained by wooden spatula.

Preparation of Smear: After smear collection, the cellular sample is evenly smeared on to the centre of the non-frosted area of the glass slide, by rotating both sides of the scrape end of the spatula in multiple clockwise swirls in contact with the slide and fixing it immediately Excessively thin or thick smears can result in false-negative reports. The smear should be visually inspected after fixation. If it does not appear satisfactory, repeat it during the same examination and submit both slides for cytological examination. Some studies have shown that two-slide cervical smears detect more abnormalities than a one-slide smear. Two smears do increase screening costs over a single-slide smear, but those costs are not double that of a single-slide examination.

Vaginal smear: Introduce an unlubricated speculum, scrape the lateral vaginal wall at the level of cervix with a spatula. The broad and flat end of Ayre’s spatula is used for this purpose. The cellular material is rapidly but gently smeared on a clean glass slide and the smears are fixed immediately. If no spatula is available a cotton swab dipped in normal saline can be used.

Vaginal pool smear: The aspiration can be performed after the introduction of unlubricated speculum. The technique allows collection of cells under direct vision from posterior fornix pool. When a speculum is not employed the pipette is gently introduced in to the vagina until resistance is encountered. It is important to compress the suction bulb during the introduction of the pipette to avoid collecting the cellular material of the lower vaginal origin. The cellular material is spread on a clean glass slide and fixed immediately.

Endometrial aspiration smear: After preliminary visualization and cleaning of cervix a sterile cannula is introduced into the uterine cavity and aspiration is then carried out with a syringe. The specimen is squirted on a clean glass slide, gently spread and rapidly fixed.

1. Discuss and add notes on the pathology of the respiratory tract. Make reference to covid-19.

Introduction

Coronavirus disease 2019 (COVID-19) is a major health concern and can be devastating, especially for the elderly. COVID-19 is the disease caused by the SARS-CoV-2 virus. Although much is known about the mortality of the clinical disease, much less is known about its pathobiology. Although details of the cellular responses to this virus are not known, a probable course of events can be postulated based on past studies with SARS-CoV. A cellular biology perspective is useful for framing research questions and explaining the clinical course by focusing on the areas of the respiratory tract that are involved. Based on the cells that are likely infected, COVID-19 can be divided into three phases that correspond to different clinical stages of the disease.

Stage 1: Asymptomatic state (initial 1–2 days of infection)

The inhaled virus SARS-CoV-2 likely binds to epithelial cells in the nasal cavity and starts replicating. ACE2 is the main receptor for both SARS-CoV2 and SARS-CoV. In vitro data with SARS-CoV indicate that the ciliated cells are primary cells infected in the conducting airways. However, this concept might need some revision, since single-cell RNA indicates low level of ACE2 expression in conducting airway cells and no obvious cell type preference. There is local propagation of the virus but a limited innate immune response. At this stage the virus can be detected by nasal swabs. Although the viral burden may be low, these individuals are infectious. The RT-PCR value for the viral RNA might be useful to predict the viral load and the subsequent infectivity and clinical course. Perhaps super spreaders could be detected by these studies. For the RT-PCR cycle number to be useful, the sample collection procedure would have to be standardized. Nasal swabs might be more sensitive than throat swabs.

Stage 2: Upper airway and conducting airway response (next few days)

The virus propagates and migrates down the respiratory tract along the conducting airways, and a more robust innate immune response is triggered. Nasal swabs or sputum should yield the virus (SARS-CoV-2) as well as early markers of the innate immune response. At this time, the disease COVID-19 is clinically manifest. The level of CXCL10 (or some other innate response cytokine) may be predictive of the subsequent clinical course. Viral infected epithelial cells are a major source of beta and lambda interferons. CXCL10 is an interferon responsive gene that has an excellent signal to noise ratio in the alveolar type II cell response to both SARS-CoV and influenza. CXCL10 has also been reported to be useful as disease marker in SARS. Determining the host innate immune response might improve predictions on the subsequent course of the disease and need for more aggressive monitoring. For about 80% of the infected patients, the disease will be mild and mostly restricted to the upper and conducting airways. These individuals may be monitored at home with conservative symptomatic therapy.

Stage 3: Hypoxia, ground glass infiltrates, and progression to ARDS

Unfortunately, about 20% of the infected patients will progress to stage 3 disease and will develop pulmonary infiltrates and some of these will develop very severe disease. Initial estimates of the fatality rate are around 2%, but this varies markedly with age. The virus now reaches the gas exchange units of the lung and infects alveolar type II cells. Both SARS-CoV and influenza preferentially infect type II cells compared to type I cells. The infected alveolar units tend to be peripheral and subpleural. SARS-CoV propagates within type II cells, large number of viral particles are released, and the cells undergo apoptosis and die. The end result is likely a self-replicating pulmonary toxin as the released viral particles infect type II cells in adjacent units. It is suspected that areas of the lung will likely lose most of their type II cells, and secondary pathway for epithelial regeneration will be triggered. Normally, type II cells are the precursor cells for type I cells. The pathological result of SARS and COVID-19 is diffuse alveolar damage with fibrin rich hyaline membranes and a few multinucleated giant cells. The aberrant wound healing may lead to more severe scarring and fibrosis than other forms of ARDS.

Recovery will require a vigorous innate and acquired immune response and epithelial regeneration. Similar to influenza, administrating epithelial growth factors such as KGF might be detrimental and might increase the viral load by producing more ACE2 expressing cells.

Elderly individuals are particularly at risk because of their diminished immune response and reduced ability to repair the damaged epithelium. The elderly also has reduced mucociliary clearance, and this may allow the virus to spread to the gas exchange units of the lung more readily.

The above comments are based on the assumption that viral entry by SARS-CoV-2 will be the same as SARS-CoV. Although it isn’t known if there are alternate receptors for viral entry. CD209L is an alternative receptor for SARS-CoV. The apical cilia on airway cells and microvilli on type II cells may be important for facilitating viral entry.

 In conclusion, COVID-19 confined to the conducting airways should be mild and treated symptomatically at home. However, COVID-19 that has progressed to the gas exchange units of the lung must be monitored carefully and supported to the best of our ability, as we await the development and testing of specific antiviral drugs.



FIGURE 1 Human alveolar type II cells infected with SARS-CoV. Human type II cells were isolated, cultured in vitro, and then infected with SARS-CoV. Viral particles are seen in double membrane vesicles in the type II cells (a) and along the apical microvilli (b). Reproduced with permission from the American Thoracic Society [8].