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The Crux

BIMONTHLY FORUM FOR THE LABORATARIANS

CONTENTS

Editorial

Cervical cancer is the second most common malignancy in women worldwide, and it remains a leading cause of cancer-related death for women in developing countries. Approximately 500,000 new cases are diagnosed each year internationally. Although Cervical cancers usually affect women of middle age or older, but it may be diagnosed in any reproductive-aged woman. A simple Pap's smear can diagnose the pre-malignant stages as well as the malignancies of the cervical tumours. Luckily enough, the process is not invasive and very cost effective. Diagnosed early, one can be assured of a 100 % cure. Clinically, the first symptom is abnormal vaginal bleeding, usually postcoital. Vaginal discomfort, malodorous discharge, and dysuria are not uncommon. The tumor grows by extending upward to the endometrial cavity, downward to the vagina, and laterally to the pelvic wall. It can invade the bladder and rectum directly. Symptoms that can evolve, such as constipation, hematuria, fistula, and ureteral obstruction with or without hydroureter or hydronephrosis, reflect local organ involvement. The triad of leg edema, pain, and hydronephrosis suggests pelvic wall involvement. The common sites for distant metastasis include extrapelvic lymph nodes, liver, lung, and bone. Early epidemiological data demonstrated a direct causal relationship between cervical cancer and sexual activity. Major risk factors observed include sex at a young age, multiple sexual partners, promiscuous male partners, and history of sexually transmitted diseases. By and large, the commonest etiological agent for cervical carcinoma that has emerged is considered to be HPV. The DISEASE DIAGNOSIS part of this issue delves deep into the clinico-diagnostic aspects of Carcinoma Cervix.

We had, in an earlier issue dealt with a simple finding of urinary crystals and how to interpret them. This article was highly appreciated as all likely causes associated with different types of crystallurias were outlined. This issue's INTERPRETATION segment beautifully defines the process, types and causes of various kinds of urinary casts.

TROUBLE SHOOTING continues probing into Malaria RDTs. A very informative article that delves into the ground realities faced during Malaria RDT testing.

BOUQUET has all the usual spices and fragrances all mixed in a nice concoction. Try it.

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DISEASE DIAGNOSIS

CERVICAL CANCER

Introduction: Cervical cancer is the second most common malignancy in women worldwide, and it remains a leading cause of cancer-related death for women in developing countries. In the United States, cervical cancer is relatively uncommon. The incidence of invasive cervical cancer has declined steadily in the United States over the past few decades; however, it continues to rise in many developing countries. The change in the epidemiological trend in the United States has been attributed to mass screening with Papanicolaou tests.

History: 400 BCE - Hippocrates: cervical cancer incurable, 1925 - Hans Hinselmann: invented colposcope, 1928 - Papanicolaou: developed Pap technique, 1941 - Papanicolaou and Trout: Pap screening, 1946 - Ayer: spatula to scrape the cervix, 1976 - Zur Hausen and Gisam: found HPV DNA in cervical cancer and warts, 1988 - Bethesda System for Pap results developed.

GROSS PICTURES OF CARCINOMA CERVIX

Frequency / International: Internationally, 500,000 new cases are diagnosed each year. Age: Cervical cancers usually affect women of middle age or older, but it may be diagnosed in any reproductive-aged woman. Types Of Cervical Malignancies: squamous cell carcinoma (about 80-85%), adenocarcinoma (about 15% of cervical cancers), adenosquamous carcinoma, small cell carcinoma, neuroendocrine carcinoma. Non-carcinoma malignancies which can rarely occur in the cervix include: melanoma, lymphoma.

Clinical: History: Because women are screened routinely, the most common finding is an abnormal Papanicolaou test result. Clinically, the first symptom is abnormal vaginal bleeding, usually postcoital. Vaginal discomfort, malodorous discharge, and dysuria are not uncommon. The tumor grows by extending upward to the endometrial cavity, downward to the vagina, and laterally to the pelvic wall. It can invade the bladder and rectum directly. Symptoms that can evolve, such as constipation, hematuria, fistula, and ureteral obstruction with or without hydroureter or hydronephrosis, reflect local organ involvement. The triad of leg edema, pain, and hydronephrosis suggests pelvic wall involvement. The common sites for distant metastasis include extrapelvic lymph nodes, liver, lung, and bone.

Physical: In patients with early-stage cervical cancer, physical examination findings can be relatively normal. As the disease progresses, the cervix may become abnormal in appearance, with gross erosion, ulcer, or mass. These abnormalities can extend to the vagina. Rectal examination may reveal an external mass or gross blood from tumor erosion. Bimanual examination findings often reveal pelvic metastasis. Leg edema suggests lymphatic/vascular obstruction from tumor. If the disease involves the liver, hepatomegaly may develop. Pulmonary metastasis usually is difficult to detect upon physical examination unless pleural effusion or bronchial obstruction becomes apparent.

Causes: Early epidemiological data demonstrated a direct causal relationship between cervical cancer and sexual activity. Major risk factors observed include sex at a young age, multiple sexual partners, promiscuous male partners, and history of sexually transmitted diseases. However, the search for a potential sexually transmitted carcinogen was unsuccessful until breakthroughs in molecular biology enabled scientists to detect viral genome in cervical cells. Strong evidence now implicates human papillomaviruses (HPVs) as prime suspects. HPV viral DNA has been detected in more than 90% of squamous intraepithelial lesions (SILs) and invasive cervical cancers compared with a consistently lower percentage in controls. Both animal data and molecular biologic evidence confirm the malignant transformation potential of papilloma virus–induced lesions. SILs are found predominantly in younger women, while invasive cancers are detected more often in women 10-15 years older, suggesting slow progression of cancer. HPV infection occurs in a high percentage of sexually active women. Most of these infections clear spontaneously within months to a few years, and only a small proportion progress to cancer. This means that other crucial factors must be involved in the process of carcinogenesis. Three main factors have been postulated to influence the progression of low-grade SILs to high-grade SILs. These include the type and duration of viral infection, with high-risk HPV type and persistent infection predicting a higher risk for progression; host conditions that compromise immunity, such as multiparity or poor nutritional status; and environmental factors such as smoking, oral contraceptive use, or vitamin deficiencies. In addition, various gynecologic factors, including age of menarche, age of first intercourse, and number of sexual partners, significantly increase the risk for cervical cancer. Human papillomavirus: HPV is a heterogeneous group of viruses that contain closed circular double-stranded DNA. The viral genome encodes 6 early open reading frame proteins (ie, E1, E2, E3, E4, E6, E7), which function as regulatory proteins, and 2 late open reading frame proteins (ie, L1, L2), which make up the viral capsid. To date, 77 different genotypes of HPV have been identified and cloned, among which, types 6, 11, 16, 18, 26, 31, 33, 35, 39, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 58, 59, 66, and 68 have the propensity to infect anogenital tissues. The HPVs that infect the human cervix fall into 2 broad categories. The low-risk types, HPV 6b and 11, are associated with low-grade SILs but are never found in invasive cancer. The high-risk types, mostly HPV 16 and 18, are found in 50-80% of SILs and in up to 90% of invasive cancers. Although less common, types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82 should also be considered carcinogenic. The major difference between the 2 types is that after infection, the low-risk HPVs are maintained as extrachromosomal DNA episomes, while the high-risk HPV genome is found integrated into the host cellular DNA. The recombination event often leaves E6 and E7 directly coupled to the viral promoter and enhancer sequences, allowing their continued expression after integration. Because E7 binds and inactivates the Rb protein while E6 binds p53 and directs its degradation, the functional loss of both TP53 and the RB genes leads to resistance to apoptosis, causing uncensored cell growth after DNA damage. This ultimately results in progression to malignancy. Human immunodeficiency virus: The role of human immunodeficiency virus (HIV) infection in the pathogenesis of cervical cancer is not fully understood. Studies have shown a higher prevalence of HPV in HIV-seropositive women than in seronegative women, and the HPV prevalence was directly proportional to the severity of immunosuppression as measured by CD4 counts. Impaired lymphocyte function has been postulated to enhance latent or subclinical HPV activity, resulting in a higher rate of persistent infection. Whether HIV has a synergistic effect on HPV infection, either by direct molecular interaction or through an indirect immunologic effect, remains unclear. Other Problems to Be Considered: Cervicitis/infection, particularly granulomatous (rare), Vaginal cancer, Metastatic cancer to cervix (rare).

Workup: Laboratory Studies: If cervical cancer is the suggested diagnosis, a Papanicolaou test should be performed. The patient should be referred to a gynecologist for colposcopy, direct biopsies, and endocervical curettage. After the diagnosis is established, a complete blood cell count and serum chemistry for renal and hepatic functions should be ordered to look for abnormalities from possible metastatic disease.

Imaging Studies: Once the diagnosis is established, imaging studies are performed for staging purposes. A routine chest radiograph should be obtained to help rule out pulmonary metastasis. CT scan of the abdomen and pelvis is performed to look for metastasis in the liver, lymph nodes, or other organs and to help rule out hydronephrosis/hydroureter. In patients with bulky primary tumor, barium enema studies can be used to evaluate extrinsic rectal compression from the cervical mass. The use of positron emission tomography (PET) scan is now recommended for patients with stage IB2 disease or higher.

Procedures: In patients with bulky primary tumor, cystoscopy and proctoscopy should be performed to help rule out local invasion of the bladder and the colon. Clinical staging protocols can fail to demonstrate pelvic and aortic lymph node involvement in 20-50% and 6-30% of patients, respectively. For that reason, surgical staging frequently is recommended. Pretreatment surgical staging is the most accurate method to determine the extent of disease. However, little evidence suggests an improvement in overall survival with routine surgical staging. Therefore, pretreatment surgical staging should be individualized after a thorough nonsurgical workup, including fine-needle aspiration of lymph nodes, has failed to demonstrate metastatic disease.

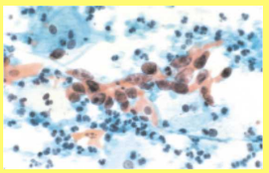
Histologic Findings: Precancerous lesions of the cervix usually are detected via Papanicolaou test. The Papanicolaou test classification system has evolved over the years. Standardized Papanicolaou test reporting being followed is given as under. Currently, cervical cytology (internationally) results are reported according to the 2001 Bethesda System.

2001 Bethesda System for Reporting Cervical Cytologic Diagnoses

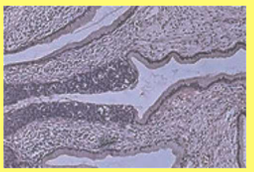
Specimen adequacy (This may be the single most important quality assurance component of the system.) Satisfactory for evaluation (note presence/ absence of endocervical/ transformation zone component). Unsatisfactory for evaluation (specify reason). Specimen rejected/not processed (specify reason). Specimen processed and examined, but unsatisfactory for evaluation of epithelial abnormality because of (specify reason).

General categorization (optional): Negative for intraepithelial lesion or malignancy. Epithelial cell abnormality. Other.

Interpretation/ result: Negative for intraepithelial lesion or malignancy. Observed organisms, such as Trichomonas, Candida, bacteria, and cellular changes consistent with herpes simplex virus, are reported. Reporting other non-neoplastic findings is optional (ie, inflammation, atrophy). Epithelial cell abnormalities. Squamous cell. Atypical squamous cells (ASC). ASC of undetermined significance (ASCUS). ASC, cannot exclude HSIL (ASC-H). Low-grade squamous intraepithelial lesion (LSIL). Encompassing: human papillomavirus/mild dysplasia/cervical intraepithelial neoplasia (CIN) 1. High-grade squamous intraepithelial lesion (HSIL). Encompassing: moderate and severe dysplasia, carcinoma in situ, CIN 2, and CIN 3. Squamous cell carcinoma. Glandular cell. Atypical glandular cells (AGC) (specify endocervical, endometrial, or not otherwise specified). AGC, favor neoplastic (specify endocervical or not otherwise specified). Endocervical adenocarcinoma in situ (AIS). Adenocarcinoma. Other.



Cytology
Squamous cell cervical carcinoma



Biopsy
Carcinoma in situ

General considerations: Complete evaluation should include Papanicolaou test with cytobrush and endocervical and endometrial samplings. If the smear result is suggestive of adenocarcinoma in situ, a cone biopsy should be performed. If the pathology still is unclear after the above workup, the patient should have dilatation and curettage. Consideration should be given to obtaining ultrasound findings that adequately define the fallopian tubes and ovaries prior to defining uterine curettage to help identify primary malignancies of these organs. Regarding invasive cervical cancer, the histology of cervical malignancy is predominantly of epithelial origin, with squamous cell carcinoma as the major group (85%). Less common histologies include adenocarcinoma, small cell carcinoma, melanoma, and lymphoma.

Staging: The TNM staging system for cervical cancer is analogous to the FIGO stage. **Stage 0** - full-thickness involvement of the epithelium without invasion into the stroma (carcinoma in situ). **Stage I** - limited to the cervix. IA - diagnosed only by microscopy; no visible lesions, IA1 - stromal invasion less than 3 mm in depth and 7 mm or less in horizontal spread, IA2 - stromal invasion between 3 and 5 mm with horizontal spread of 7 mm or less, IB - visible lesion or a microscopic lesion with more than 5 mm of depth or horizontal spread of more than 7 mm, IB1 - visible lesion 4 cm or less in greatest dimension, IB2 - visible lesion more than 4 cm. **Stage II** - invades beyond cervix. IIA - without parametrial invasion, but involve upper 2/3 of vagina, IIB - with parametrial invasion. **Stage III** - extends to pelvic wall or lower third of the vagina. IIIA - involves lower third of vagina, IIIB - extends to pelvic wall and/or causes hydronephrosis or non-functioning kidney. **IVA** - invades mucosa of bladder or rectum and/or extends beyond true pelvis. **IVB**- distant metastasis.

Treatment: Medical Care: The treatment of cervical cancer varies with the stage of the disease. For early invasive cancer, surgery is the treatment of choice. In more advanced cases, radiation combined with chemotherapy is the current standard of care. In patients with disseminated disease, chemotherapy or radiation provides symptom palliation. Stage 0: Treatment options for stage 0 cancer include loop electrosurgical excision procedure (LEEP), laser therapy, conization, and cryotherapy. Stage IA: The treatment of choice for stage IA disease is surgery—total hysterectomy, radical hysterectomy, and conization are accepted procedures. According to international guidelines, pelvic radiation therapy is now a category 1 recommendation for women with stage IA disease and negative lymph nodes after surgery who have high-risk factors, including large primary tumor, deep stromal invasion and/or lymphovascular space invasion. Stage IB or IIA: For patients with stage IB or IIA disease, treatment options are either combined external beam radiation with brachytherapy or radical hysterectomy with bilateral pelvic lymphadenectomy. Radical trachelectomy with pelvic lymph node dissection is appropriate for fertility preservation in women with stage IA2 disease, and those with stage IB1 disease whose lesions are >2 cm. Most retrospective studies have shown equivalent survival rates for both procedures, although such studies usually are flawed due to patient selection bias and other compounding factors. However, a recent study showed identical overall and disease-free survival rates. Quality-of-life data, particularly in the psychosexual area, is relatively scant. Postoperative radiation to the pelvis decreases the risk of local recurrence

in patients with high-risk factors (positive pelvic nodes, positive surgical margins, and residual parametrial disease). A randomized trial showed that patients with parametrial involvement, positive pelvic nodes, or positive surgical margins benefit from a postoperative combination of cisplatin-containing chemotherapy and pelvic radiation. Stage IIB-IVA: For locally advanced cervical carcinoma (stages IIB, III, and IVA), radiation therapy was the treatment of choice for many years. However, the results from large, well-conducted, prospective randomized clinical trials demonstrated a dramatic improvement in survival with the combined use of chemotherapy and radiation. Consequently, the use of cisplatin-based chemotherapy in combination with radiation has become the standard of care for patients with locally advanced cervical cancer. Radiation therapy begins with a course of external beam radiation to reduce tumor mass to enable subsequent intracavitary application. Brachytherapy is delivered using afterloading applicators that are placed in the uterine cavity and vagina. Stage IVB and recurrent cancer: These patients are treated with chemotherapy. For many years, single-agent cisplatin represented the standard of care. Recently, the combined use of cisplatin and topotecan was shown to significantly improve survival compared with single-agent cisplatin. Palliative radiation is often used on an individualized basis to control bleeding, pelvic pain, or urinary or partial large bowel obstructions from pelvic disease. Special effort should be made to ensure comprehensive palliative care, including adequate pain control for these patients.

Surgical Care: Carcinoma in situ (stage 0) is treated with local ablative measures such as cryosurgery, laser ablation, and loop excision. Hysterectomy should be reserved for patients with other gynecologic indications to justify the procedure. After local treatment, these patients require lifelong surveillance. Palliative radiation often is used individually to control bleeding, pelvic pain, or urinary or partial large bowel obstructions from pelvic disease. Invasive procedures such as nephrostomy or diverting colostomy sometimes are performed in this group of patients to improve their quality of life. The standard treatment for microinvasive disease (stage IA) is total hysterectomy. Lymph node dissection is not required if the depth of invasion is less than 3 mm and no lymphovascular invasion is noted. Selected patients with stage IA1 disease but no lymphovascular space invasion who desire to maintain fertility may have a therapeutic conization with close follow-up, including cytology, colposcopy, and endocervical curettage. Patients with medical comorbidities who are not surgical candidates can be successfully treated with radiation. Total pelvic exenteration may be considered in patients with an isolated central pelvic recurrence.

Consultations: The treatment of cervical cancer frequently requires a multidisciplinary approach involving a **gynecologic oncologist, radiation oncologist, and medical oncologist. Diet:** Proper nutrition is important for patients with cervical cancer. Every attempt should be made to encourage and provide adequate oral food intake. Nutritional supplements such as Ensure or Boost are used when patients have had significant weight loss or cannot tolerate regular food due to nausea caused by radiation or chemotherapy. In patients with severe anorexia, appetite stimulants such as megestrol (Megace) can be prescribed. For patients who are unable to tolerate any oral intake, percutaneous endoscopic gastrostomy tubes are placed for nutritional supplementation. In patients with extensive bowel obstruction as a result of metastatic cancer, hyperalimentation sometimes is used. **Medication:** Chemotherapy should be administered in conjunction with radiation therapy to most patients with stage IB (high risk) to IVA. Cisplatin is the agent used most commonly, although 5-fluorouracil also is used frequently. For patients with metastatic disease, cisplatin remains the most active agent. Topotecan, ifosfamide, and paclitaxel also have significant activity in this setting. The combination of topotecan and cisplatin improves overall survival. However, acute toxicities are also increased.

Follow-up: Deterrence/Prevention: Screening of cervical cancer: For many years, the standard method for cervical cancer screening has been the Papanicolaou test. Retrospective data have shown that screening with a Papanicolaou test reduces the incidence of cervical cancer by 60-90% and the death rate by 90%. The false-negative rate of a Papanicolaou test is 20%, which mostly results from sampling error. Physicians can reduce sampling error by ensuring adequate material is taken from both the endocervical canal and the ectocervix. Smears without endocervical or metaplastic cells must be repeated. Upon physical examination, suspicious or grossly abnormal cervical lesions should undergo biopsy regardless of cytologic findings. Recently, new technologies have become available. Limited information is available regarding their sensitivity and specificity (compared with the conventional Papanicolaou test). Whether these new methods improve survival, compared with the conventional Papanicolaou test, is unknown. Since its introduction more than 50 years ago, the use of the Papanicolaou test for cervical screening has reduced mortality by 70%. Nonetheless, the mortality rate in the United States has remained relatively constant during the last 25 years. One of the factors to explain this has been described as limitations of the traditional Papanicolaou test method itself. The limitations of the conventional Papanicolaou test include limited sensitivity (51%) and a significant proportion of inadequate specimens. In addition, accurate interpretation of conventional Papanicolaou tests are often compromised by the presence of artifacts (such as blood, mucus, obscuring inflammation, scant cellular material, and air-drying artifact). Thin Prep test: The ThinPrep test samples are collected the same way as the conventional Papanicolaou test. However, the specimen is placed in a preservative solution rather than on a slide. An automated processor prepares the sample and makes a uniform slide for review. Mucus and blood are removed in the process. The ThinPrep Papanicolaou test was approved in 1996 as an alternative to the traditional conventional smear. HPV testing: The Hybrid Capture II HPV test was approved by the Food and Drug Administration (FDA) as a new approach for cervical cancer in 2003. This test is indicated for women aged 30 years and older, in conjunction with the Papanicolaou test. If both tests are negative, then the next Papanicolaou test can be delayed for 3 years. The HPV test is also useful to interpret equivocal results from a Papanicolaou test. If a women has an ASCUS Papanicolaou test result and a positive HPV test, then additional workup with a colposcopy is indicated. **Screening recommendations are as follows:** Internationally accepted recommendations are that all women should begin screening for cervical cancer approximately 3 years after they begin to have vaginal intercourse, but no later than age 21. Beginning at age 30, women who have had 3 consecutive normal Papanicolaou test results may get screening every 2-3 years. Women with high risk factors (DES exposure, HIV infection, or other immunodeficiencies) should continue yearly screening. Another option for women aged 30 years and older is to get screened every 3 years with the conventional- or liquid-based Papanicolaou test plus HPV DNA test. It is also recommended that women aged 70 years and older with 3 or more normal consecutive Papanicolaou test results and no abnormal Papanicolaou test results within the last 10 years may choose to stop having cervical cancer screening. The international Gynaecologic societies recommend against routinely screening women older than age 65 cancer if they have had adequate recent screening with normal Pap smears and are not otherwise at high risk for cervical cancer. Women who have had a total hysterectomy may stop having cervical cancer screening. Exceptions are those who had a hysterectomy due to cervical carcinoma (or preinvasive changes) and women who had a hysterectomy without removal of the cervix. **Prevention:** Several measures are effective to prevent HPV infection and hence prevent cervical cancer. Sexual abstinence, Barrier protection and/or spermicidal gels during sexual intercourse, Vaccination: Evidence suggests that HPV vaccines prevent HPV infection. A vaccine for HPV, Gardasil, is approved by the FDA for girls and women 9 to 26 years of age for prevention of cervical cancer caused by HPV types 6, 11, 16, and 18.

TROUBLESHOOTING

MALARIA - RDTs

.....Continued from earlier issue.

Problems associated with the usage of Malaria RDT's.

RDT's retrieved from cold storage (2-8°C) must be allowed to come to room temperature (ambient temperature, in case of field conditions) before the pouches are opened and the test used. When specimen is added to a cold device, it attacks a 'moisture rush' thereby altering the migration properties of the membrane. If specimen is added to 'cold' devices the blood flow is usually slowed down affecting the background clearance and visualization of test results; especially of specimen containing low target antigen concentration. At lower temperatures the antigen-antibody binding is less than optimum, leading to loss in sensitivity and resultant signals. Use of specimen transfer devices such as loops and straws are extremely simple to use. However, to ensure accuracy and precision of sample delivery, user training has to be imparted to build usage competence with actual users. The end point of a Malaria RDT reaction is qualitative. Its interpretation has an element of personal subjectivity, that introduces variability. What appears as a weak positive to our reader may well be negative for another. Usually "tie break"method or "best of 3" is the best way to resolve interpretative subjectivity. User training, hands on experience in reading and interpreting RDT end points must be assessed for consistency as a prerequisite. This is especially relevant when samples used during evaluation are low analyte concentrations, nearing the detection limits of RDT's are being used that generate very low signal intensities.

Alternative approach to Laboratory Quality Assurance of Malaria RDT's

For large scale comparative trials of malaria RDT's, availability of standardized, fresh and well characterized clinical specimen is essential. However, achieving this has posed significant challenges due to variability linked to specimen collection, preservation and logistics involved. To overcome these challenges, centralized evaluations with regards to the performance evaluation of malaria RDT's have used alternative methods. These methods have involved alternate materials such as cultured P. falciparum parasites at different parasitic densities, wild type P. falciparum and P. vivax specimen etc. These samples have been diluted and cryo-preserved before use. During evaluation the panels so prepared have been thawed and used to assess the performance of the malaria RDT's with regards to their performance especially towards sensitivity, specificity and other characteristics.

Effect of Clinical Specimen Quality on Performance of Malaria RDT's.

Malaria RDT's are designed and standardized primarily for testing specimen obtained from fresh capillary whole blood in field conditions, and blood correctly collected through veni puncture in laboratory settings. Venous blood collected in appropriate anticoagulants, when stored at 2-8^0 C may be stable for up to 72 hours provided they are not contaminated. Unhindered sample flow is at the heart of the performance of a malaria RDT. When the sample does not flow on the Malaria RDT, blood clearance on the nitrocellulose membrane is affected. Poor clearance affects visualization of the test results and also the sensitivity and specificity of the RDT.

The following aspects of sample quality affect the functioning of malaria RDT's. Cold blood samples can flow differently from those stored at R.T. Stored blood can loose target antigen activity. Early lysis, protein coagulation, presence of artefacts inhibit the flow of blood specimen on RDT's. Freeze thaw accelerates target antigen denaturation and alters blood flow properties on malaria RDT's, hampering their performance. In some instances, for some products improvement in RDT sensitivity has been reported, owing to target antigen release through parasite lysis. Performance of RDT's using venous blood specimen and archived blood specimen can differ from performance obtained with fresh finger prick blood.

Effect of Prepared whole blood controls on performance of Malaria RDT's.

Though Malaria RDT's are essentially designed and optimized to work with capillary finger prick whole blood samples, for large scale laboratory based trials it may become necessary to procure well characterized veni puncture blood and use diluted or archived whole blood for longitudinal studies. Preparation of serial parasite dilution necessitates mixing parasitised blood into well characterized parasite negative whole blood. The preparation of serial dilution can affect quality of the diluted blood due to: Inadequate mixing. Blood type incompatibility. Freeze thawing during storage and use. Cell lysis due to use of small bore pipettes for dispensing samples. The method of preparation of whole blood controls can affect the blood flow on the RDT's, greatly influencing on the thresh holds of sensitivity, specificity and the validity of the Malaria RDT's.

Effect of Artificial panels and reference materials on the performance of RDT's.

It is well known that the production of target antigen varies with the stage of the parasite development. Yet at present there is insufficient information on the relationship of target antigen concentration and parasite density. In vivo, during the malarial infection as the parasitaemia increases, there is a sustained build up of the target antigens in the blood. On the other hand in vitro, when the parasite is grown in culture systems the dynamics of the production of the target antigen is completely different. This is probably due to the dissimilar environment for growth between the cultured and the wild parasites. Thus, the relationship between parasite density and target antigen activity varies significantly between wild type parasites and cultured parasites. This fact introduces a disconnect between "parasitaemia" and "Target antigen concentration". When reference material from these two systems are used for assessing performance of RDT's, the reference material prepared from the "cultured parasites"would not accurately reflect the target antigen concentrations as may be present in the wild type parasites, even for synchronous stage of parasitaemia or stage of development.

Importance of Clinical Samples

Target antigen sequences vary from region to region for the Malaria parasites. Especially for Pf HRP-II, wide diversity in Pf HRP-II sequences both within and between countries have been reported. Variation in the number and combination of repeats within the Pf HRP II sequences have been shown to affect the sensitivity of Malaria RDT's. Additionally various isolates also differ in terms of: Antigen expression. Structural variations. Variations in parasite density versus target antigen concentration. The above differences introduce variations in apparent RDT sensitivities from region to region. These factors also make setting lower detection limits for Malaria RDT's in terms of Parasite density practically and biologically incredible. In fact it makes strong case for well designed and executed local level performance evaluation of products even stronger.

Commutability of Reference Materials & Standards

The fundamental goal of laboratory medicine remains that "results for patient samples should be comparable independent of the medical laboratory that produced the results" Cummutability of a Control material is its ability to have inter assay properties comparable to properties demonstrated by authentic clinical samples. Commutability must be x

validated and demonstrated amongst all the methods that will use the material including the reference test procedure. Until commutability of a reference material is not established, results from various methods cannot be legitimately compared. Non commutability of reference materials can be caused by Matrix alteration or a on native analyte. The Matrix effect or Matrix bias is caused by the differences in sample Matrix of the reference material and the native clinical samples. Non native forms of the target analytes, can produce a different measurement signal than expected from the native forms of analyte. Ideally the sample matrix includes all components of a material system except the analyte itself. Test calibration with reference materials that are not commutable can cause poorer rather then improved agreement of results among methods for native samples. Poor commutability of reference materials cause grave difference in apparent product performance and introduce bias in measurement of analytes, compromising comparability of product performance.

Setting Standards for Sensitivity and Performance

Since Malaria RDT's detect the appropriate 'target antigens' and not the 'parasite' per se, “parasite antigen concentrations” could be a more appropriate benchmark to be used to set the lower limits of detection for Malaria RDT's. Determination of the relationship between target antigen concentration to parasite density would be a critical step towards this goal. There is an urgent need to make available commutable reference panels that are well characterized in terms of target antigen concentration and their antigenic structure for the objective evaluation of the performance of malaria RDT's and for their unbiased comparability.

Summary

Various methods have been devised and are in the process of evolution with regards to validating the sensitivity, specificity and performance of Malaria RDT's. Each method has the potential to significantly alter the specimen characteristics in ways that is detrimental to the performance characteristics of malaria RDT's in unpredictable ways. Preparation and availability of commutable Reference material and their wide availability to manufacturers, regulators and users, by setting a common and consistent “reference Benchmark” could lead to the desired outcome for improvement in quality, consistency, performance and deliverance of “better Malaria RDT's” to the users.

Till such time: Well designed and executed field / laboratory evaluations of Malaria RDT's. Performed by well trained personnel. Using clinical / well characterized specimen bank. At the user level.

Perhaps remains the most appropriate, effective and practical method for assessing the performance of Malaria RDT's and their appropriateness for use in each setting.

BOUQUET

In Lighter Vein

A man walks into the front door of a bar. He is obviously drunk, and staggers up to the bar, seats himself on a stool and, with a belch, asks the bartender for a drink. The bartender politely informs the man that it appears that he has already had plenty to drink, he could not be served additional liquor at this bar, and could a cab be called for him?

The drunk is briefly surprised, then softly scoffs, grumbles, climbs down off the bar stool and staggers out the front door. A few minutes later, the same drunk stumbles in the SIDE door of the bar. He wobbles up to the bar and hollers for a drink. The bartender comes over and, still politely - but more firmly, refuses service to the man due to his inebriation, and again offers to call a cab. The drunk looks at the bartender for a moment angrily, curses, and shows himself out the side door, all the while grumbling and shaking his head.

A few minutes later, the same drunk bursts in through the back door of the bar. He plops himself up on a bar stool, gathers his wits and belligerently orders a drink. The bartender comes over and emphatically reminds the man that he is clearly drunk, will be served no drinks, and either a cab or the police will be called immediately.

The surprised drunk looks at the bartender, and in hopeless anguish, cries "Man! How many bars do you work at?”

“Yesterday, scientists revealed that beer contains small traces of female hormones. To prove their theory, the scientists fed 100 men 12 pints of beer and observed that 100% of them gained weight, talked excessively without making sense, became emotional, couldn't drive, and refused to apologize when wrong. No further testing is planned.”

Two 80 year old men sat talking over the weather and the latest in medical science, and such, when one brings up the latest male medical miracle, Viagra.

The other wasn't familiar with Viagra and asked the first man what it was for. The first man said, "It's the greatest thing I've ever known. The Fountain of Youth!! Makes you feel like a man of 30."

The second then asked, "Can you get it over the counter?"

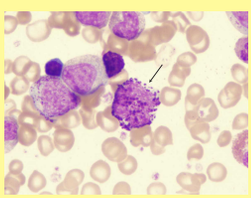
“You probably could, if you took 2 pills", said the first man.

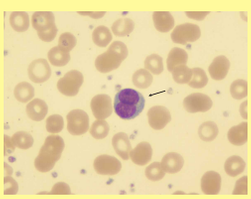
Wisdom Whispers

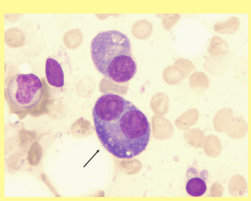
- “He that would have the fruit, must climb the tree."
- "He is not a bad driver who knows how to turn."
- "Open confession is good for the soul."
- "The higher the ape climbs the more he shows his rump."
- "There is no such thing as a free lunch."
- "Wear not out your welcome."
- "If you ever need a helping hand, you'll find one at the end of your arm."
- "He has too many lice to feel an itch."
- "A triple rope is not easily broken."

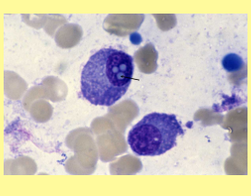
Brain Teasers

Try to identify the marked cells in the pictures.

1. 

2. 

3. 

4. 

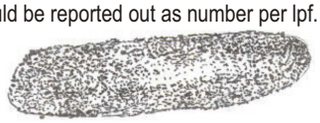
Answers: Fig1. Basophil Fig2. Bilobed lymphocyte, Fig 3. Binucleated Plasma cell, Fig. 4 Duthcer body.

INTERPRETATION

URINARY CASTS

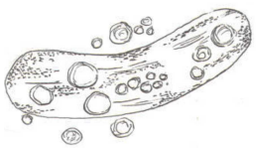
GRANULAR CAST AND ITS CLINICAL SIGNIFICANCE.

Granular casts are acellular casts and are generally thought to be a stage in the degeneration process of cellular casts. The size of the granules in this cast varies resulting in two general categories: fine and coarse granular casts. Neither is more significant than the other, therefore the size of granules are not differentiated and these casts are simply reported out as granular casts per lpf. It is not uncommon to find granular casts accompanying hyaline casts in times of physical exertion, emotional stress, dehydration, or heat stress. It is the second most commonly occurring cast in urine sediment. When viewed in the brightfield microscope, these casts appear colorless or yellow in appearance. If you observe a hyaline cast with several obvious granules, but the cast is predominately hyaline, then report the cast as the hyaline type. This cast has a high refractive index and is most often observed in the cigar shape. This cast will take on a variety of colors dependent upon the staining material. a. Sternheimer-Malbin stain causes dark blue coloration of the granules. b. Bilirubin will stain the cast a yellow-orange color. c. Phenazopyridium produces a orange-red color. This cast can been reported to have been confused with the hemoglobin cast. It can be confused with the bacterial cast. The bacterial cast can be differentiated with Gram's stain. The granular cast should be reported out as number per lpf. This cast is observed in all renal disorders, stress, and during the recovery phase of acute kidney failure.



FATTY CAST AND ITS CLINICAL SIGNIFICANCE.

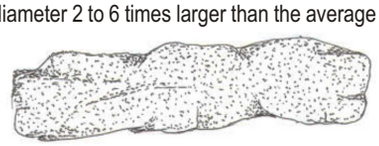
This cast may contain fat oval bodies or fat globules or both (see Figure below) and when present is pathologically significant. It is characterized by a high refractive index, stains with Sudan III or IV (if triglycerides are present) and the polarized microscope demonstrates the Maltese cross phenomenon (if cholesterol and its esters are present). The following may be observed when fatty casts are present: [1] variable size fat globules within the cast. [2] will NOT take up Sternheimer-Malbin stain. [3] cast matrix will be either hyaline or granular in type. [4] proteinuria is present. [5] variable size free-floating fat globules in the "neighborhood". [6] strongly positive protein test on the reagent test strip or 3% SSA test. [7] the urine strongly foams when shaken due to increased albuminuria.



The presence of these casts indicates the following type of renal pathology: (1) nephrotic syndrome, (2) lipid necrosis, (3) diabetic neuropathy, (4) lupus neuropathy, (5) any chronic renal disorder, and (6) renal tubular cell death. Report out this cast as numbers per lpf.

WAXY CAST, HOW TO RECOGNIZE IT, AND ITS CLINICAL SIGNIFICANCE.

This is an acellular cast with a very high refractive index. Its presence in a urine specimen is an indicator of renal tubular damage, severe stasis and is a very serious pathological finding. These casts are associated with chronic renal failure, nephrotic syndrome, diabetic neuropathy, renal allograft rejection, and renal amyloidosis. In the brightfield microscope, the waxy cast is homogeneously smooth in appearance, has parallel sides with sharp margins (in which cracks, fissures, convolutions, and notches can be observed), ends that are often blunt and a broken off appearance, and will appear to have thickened areas. The color of the waxy cast in unstained sediment is from colorless to gray to yellowish. If stained with Sternheimer-Malbin stain, the waxy cast appears pink. Because waxy casts appear in urine specimens with a serious pathology, these casts are often appear with diameter 2 to 6 times larger than the average casts. These casts are broad casts or renal failure casts.



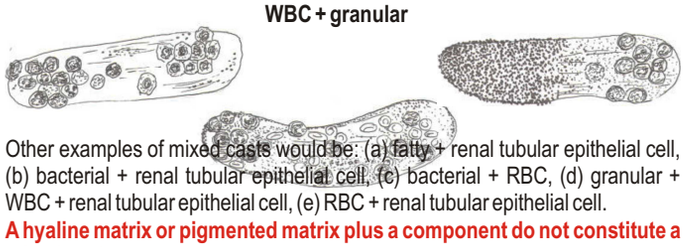
"MIXED" CAST AND ITS CLINICAL SIGNIFICANCE.

A mixed cast is one that has more than one component. It is not unusual for more than one component to be incorporated into a Tamm-Horsfall matrix. Clinical significance of finding mixed casts will correlate to that described for a specific constituent. Mixed casts may be any one of the following components as shown in the following examples.

WBC + renal tubular epithelial cell

WBC + RBC

WBC + granular



Other examples of mixed casts would be: (a) fatty + renal tubular epithelial cell, (b) bacterial + renal tubular epithelial cell, (c) bacterial + RBC, (d) granular + WBC + renal tubular epithelial cell, (e) RBC + renal tubular epithelial cell.

A hyaline matrix or pigmented matrix plus a component do not constitute a mixed cast.

BACTERIAL CAST AND ITS CLINICAL SIGNIFICANCE.

Bacterial casts may be expected to occur whenever a patient is diagnosed with pyelonephritis. The bacteria are often difficult to discern within the cast matrix and it is not unusual for a bacterial cast to be reported out as a granular cast. The following information will help to identify the bacterial cast. (a) Bacteria should be in the "neighborhood". (b) WBC should be in the "neighborhood" and may be present in the cast, in which case, the cast is a mixed cast. Because of the responsiveness of neutrophils to bacterial presence, few true bacterial casts are observed. (c) WBC casts may be present. (d) The protein, nitrite and leukocyte esterase pads on the reagent strip should be positive. Note: Performing a Gram stain may facilitate recognition of the bacterial cast.

PIGMENTED CAST AND ITS CLINICAL SIGNIFICANCE.

The pigmented cast is usually a hyaline cast that has a distinctive coloration form (1) bilirubin, (2) hemoglobin, (3) myoglobin, (4) phenazopyridium, or (5) any strongly colored medication. Ordinary urine pigments do not stain casts. If the pigment is due the hemoglobin, this could be an indicator of a transfusion reaction or hemolytic anemia. If the pigment is due to myoglobin, then muscle trauma or acute renal failure may be the cause. If bilirubin is present, not only will the casts be stained, but also the cellular elements. Bilirubin is an indicator of hepatitis. Strongly colored medications such as phenazopyridium is an indicator that a treatment process is in progress and may not be considered to be clinically significant.

"TELESCOPED" URINE SEDIMENT.

Telescoped urine is a term that describes the appearance of all types of casts and any other pathological components in the urine sediment. It has been used to describe the sediment of patients diagnosed with acute glomerulonephritis. Its original meaning described the simultaneous appearance of WBC's and RBC's in urine. The term now includes the appearance of casts, including broad casts, waxy casts, and oval fat bodies.

"ATHLETIC PSEUDO-NEPHRITIS".

This is a physiological, transient condition that occurs when strenuous activity is followed by a release of hyaline and granular casts in the urine. Once the stress is relieved, the condition disappears.

"BROAD" CASTS.

Broad casts occur when the flow of urine in the lumen of the tubules becomes very compromised. Formation usually occurs within dilated or atrophied distal tubules and the larger collecting tubules. Their presence is an indicator of a poor prognosis. They may be of any type of cast, but granular and waxy types are more often observed. Broad casts will range from two to six times larger than the typical cast. These casts are sometimes called renal failure casts.

CRYSTAL CASTS AND THEIR CLINICAL SIGNIFICANCE.

Crystal casts occur when solutes precipitate in the lumen of the renal tubules and become trapped in the hyaline cast matrix. It is the consensus of most professionals that these casts have no clinical significance. The two most commonly encountered crystal-type casts are calcium oxalates and sulfonamides, with uric acid crystals in third place. Casts with amorphous urates have been reported. Before reporting out a crystal cast, be sure that you confirm the presence of a protein matrix and that these are not crystals aligned along a sticky strand of mucus. Because crystal casts may cause irritation in the tubules, bleeding may occur. Some degree of hematuria may accompany such casts in urine.

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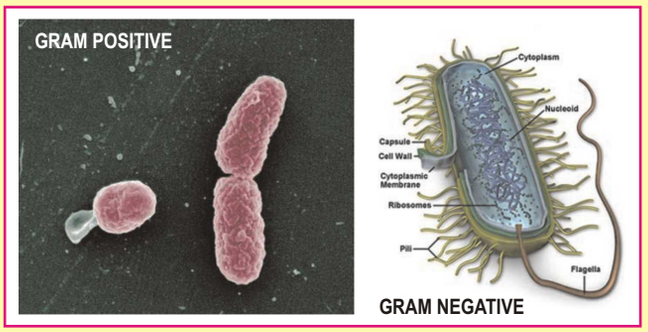

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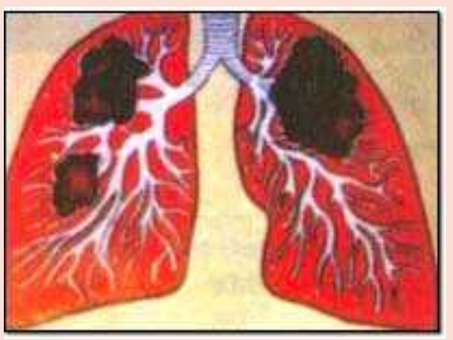

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