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The Crux

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CONTENTS

Editorial

Which disease is also known as Bang's disease, Gibraltar fever, Malta fever, Maltese fever, Mediterranean fever, rock fever, or undulant fever and has also in the past been known as Brucelliasis, Bruce's septicemia, continued fever, Crimean fever, Cyprus fever, febris melitensis, febris undulans, goat fever, melitensis septicemia, melitococcosis, milk sickness, mountain fever, Neapolitan fever, slow fever. Yes, you have guessed it right, it is Brucellosis. Brucellosis is believed to be an ancient disease that was described more than 2000 years ago by the Romans. Bruce first isolated *Brucella melitensis* in 1887. Since then, brucellosis has become an emerging disease in many parts of the world. Brucellosis is a worldwide zoonosis caused by infection with the bacterial genus Brucella. These organisms, which are small aerobic intracellular coccobacilli, localize in the reproductive organs of host animals, causing abortions and sterility. They are shed in large numbers in the animal's urine, milk, placental fluid, and other fluids. Exposure to infected animals and animal products causes brucellosis in humans. The interest in brucellosis has been increasing because of the growing phenomena of international tourism and migration, in addition to the potential use of Brucella as a biological weapon. Familiarity with the manifestations of brucellosis and the optimal laboratory studies is essential for physicians to recognize this re-emerging zoonosis. DISEASE DIAGNOSIS section of this issue highlights all clinico-diagnostic aspects of Brucellosis.

The overflow from the INTERPRETATION portion of CD4 counts and HIV/ AIDS management has spilt into present issue. It's an important topic as the disease has spread its tentacles like wildfire globally. No cure is available as on date. Only prevention is the tool. Good management can decrease morbidity and delay mortality.

We have in the past written about Quality Assurance in Haematology, Clinical Biochemistry, ELISA/ CLIA, Microbiology/ bacteriology etc. This issue takes up QA aspects as elated to Urine Examination and Reporting. TROUBLE SHOOTING part in this issue takes up a simple investigation like urine examination and lays threadbare all QA aspects as related to it.

BOUQUET is metamorphosing repeatedly. Instead of photomicrographs, this issue presents hepatic pathology as seen grossly by the naked eyes. Other components haven't gone missing. Peep inside please.

DISEASE DIAGNOSIS

BRUCELLOSIS

Introduction

Synonyms: Brucellosis, also called Bang's disease, Gibraltar fever, Malta fever, Maltese fever, Mediterranean fever, rock fever, or undulant fever. Brucellosis is believed to be an ancient disease that was described more than 2000 years ago by the Romans. Bruce first isolated Brucella melitensis in 1887. Since then, brucellosis has become an emerging disease in many parts of the world. Brucellosis is a worldwide zoonosis caused by infection with the bacterial genus Brucella. These organisms, which are small aerobic intracellular coccobacilli, localize in the reproductive organs of host animals, causing abortions and sterility. They are shed in large numbers in the animal's urine, milk, placental fluid, and other fluids. Exposure to infected animals and animal products causes brucellosis in humans. The global burden of human brucellosis remains enormous; it causes more than 500,000 infections per year worldwide. The interest in brucellosis has been increasing because of the growing phenomena of international tourism and migration, in addition to the potential use of Brucella as a biological weapon. Familiarity with the manifestations of brucellosis and the optimal laboratory studies is essential for physicians to recognize this re-emerging zoonosis. Recently, *B melitensis, Brucella abortus,* and *Brucella suis* have been completely sequenced, which will help improve our understanding of the complex pathogenesis and the diverse manifestations of this complex disease. The traditional classification of Brucella species is based largely on the preferred hosts.

The 7 Currently Recognized Brucella Species are given in the table below:

Organism	Animal Reservoir	Geographic Distribution
B. melitensis	Goats, sheep, camels	Mediterranean, Asia, Latin America, parts of Africa and some southern European countries
B. abortus	Cows, buffalo, camels, yaks	Worldwide
B. suis	Pigs (biotype 1-3)	South America, South East Asia, United States
Brucella canis	Canines	Cosmopolitan
Brucella ovis	Sheep	No known human cases
Brucella neotomae	Rodents	Not known to cause human disease
Brucella pinnipediae and *Brucella cetaceae*	Marine animals, minke whales, dolphins, seals	Recent case reports describing some human cases (mainly neurobrucellosis)

Among the 4 Brucella species known to cause disease in humans (*B. abortus, B. melitensis, B. canis, B. suis*), *B. melitensis* is thought to be the most virulent and causes the most severe and acute cases of brucellosis. *B. melitensis* is also the most prevalent worldwide. A prolonged course of illness, often associated with suppurative destructive lesions, is associated with *B. suis* infections. *B. abortus* is associated with mild-to-moderate sporadic disease that rarely causes complications. *B. canis* infection has a disease course that is indistinguishable from *B. abortus* infection. *B. canis* infection has an insidious onset, causes frequent relapses, and does not commonly cause chronic brucellosis. *B. pinnipediae* and *.B cetaceae* are distinctive species that typically affect marine animals; however, these strains were recently described to cause disease in humans, mainly neurobrucellosis. Definitive diagnosis of brucellosis is based on culture, serologic techniques, or both. Clinically, identification to the genus level is adequate to initiate therapy, and the type of Brucella species involved does not alter the therapeutic agents used; however, speciation is necessary for epidemiologic surveillance and requires more detailed biochemical, metabolic, and immunologic testing.

History and nomenclature

The disease now called brucellosis, under the name "Malta fever", first came to the attention of British medical officers in Malta during the Crimean War in the 1850s. The causal relationship between organism and disease was first established by Dr. David Bruce in 1887. In 1897, Danish veterinarian Bernhard Bang isolated *Brucella abortus* as the agent, and the additional name "Bang's disease" was assigned. In modern usage, "Bang's disease" is often shortened to just "Bangs" when ranchers discuss the disease or vaccine. Maltese doctor and archaeologist Sir Themistocles Zammit earned a knighthood for identifying unpasteurized milk as the major source of the pathogen in 1905, and it has since become known as Malta Fever. In cattle this disease is also known as contagious abortion and infectious abortion. The popular name "undulant fever" originates from the characteristic undulance (or "wave-like" nature) of the fever which rises and falls over weeks in untreated patients. In the 20th century, this name, along with "brucellosis" (after Brucella, named for Dr Bruce), gradually replaced the 19th century names "Mediterranean fever" and "Malta fever". In 1989, neurologists discovered neurobrucellosis, a neurological involvement in brucellosis.

Pathophysiology

Brucella species have a unique ability of invading both phagocytic and nonphagocytic cells and surviving in the intracellular environment by avoiding the immune system in different ways, explaining why brucellosis is a systemic disease and can involve almost every organ system. After ingestion by phagocytes, approximately 15-30% of Brucella organisms survive. In polymorphonuclear or mononuclear phagocytic cells, the bacteria use numerous mechanisms to avoid or suppress bactericidal responses. Based on animal models, the lipopolysaccharide (LPS; smooth in *B. melitensis, B. abortus*, and *B. suis* and rough in *B. canis*) was found likely to play a substantial role in intracellular survival, perhaps because of adenine and guanine monophosphate production, which inhibits phagosomal fusion and oxidative burst activity. In addition, Brucella species have relatively low virulence, toxicity, and pyrogenicity, making them a poor inducer of some inflammatory cytokines such as tumor necrosis factor (TNF) and interferons. Also, the bacteria do not activate the alternative complement system. Finally, it is thought to inhibit programmed cell death. After replication in the endoplasmic reticulum, the brucellae are released with the help of hemolysins and induced cell necrosis. Susceptibility to intracellular killing differs among species, with *B. abortus* readily killed and *B. melitensis* rarely affected; this might explain the differences in pathogenicity and clinical manifestations in human cases of brucellosis.

Frequency

International: Brucellosis causes more than 500,000 infections per year worldwide. The heaviest disease burden lies in countries of the Mediterranean basin and Arabian Peninsula, and the disease is also common in India, Mexico, and South and Central America. Although some countries have effectively controlled brucellosis, new areas of human brucellosis have emerged in areas such as central Asia. Disease

incidence and prevalence rates vary widely among nations. Because of variable reporting, true estimates in endemic areas are unknown. Incidence rates of 1.2-70 cases per 100,000 people are reported.

Mortality/Morbidity: Human brucellosis carries a low mortality rate (<5%), mostly secondary to endocarditis, which is a rare complication of brucellosis. However, brucellosis can cause chronic debilitating illness with extensive morbidity.

Sex: Worldwide, brucellosis is more common in males than in females, with a ratio of 5:2-3 in endemic areas.

Age: In multiple large series, persons in their third to fifth decades of life were most commonly affected. Brucellosis in children comprises 3-10% of reported cases worldwide, with a heavier burden in endemic areas. Elderly persons typically develop chronic brucellosis.

Clinical

History: Symptoms of brucellosis are protean in nature, and none is specific enough to support the diagnosis. Fever is the most common symptom and sign of brucellosis, occurring in 80-100% of cases. It is intermittent in 60% of patients with acute and chronic brucellosis and undulant in 60% of patients with subacute brucellosis. Fever can be associated with a relative bradycardia. Fever of unknown origin (FUO) is a common initial diagnosis in patients in areas of low endemicity. It is associated with chills in almost 80% of cases. Constitutional symptoms of brucellosis include anorexia, asthenia, fatigue, weakness, and malaise and are very common (>90% of cases).

Bone and joint symptoms include arthralgias, low back pain, spine and joint pain, and, rarely, joint swelling. These symptoms affect as many as 55-80% of patients. Neuropsychiatric symptoms of brucellosis are common despite the rare involvement of the nervous system. Headache, depression, and fatigue are the most frequently reported neuropsychiatric symptoms. Gastrointestinal symptoms, present in 50% of patients, include abdominal pain, constipation, diarrhea, and vomiting. Neurologic symptoms of brucellosis can include weakness, dizziness, unsteadiness of gait, and urinary retention. Symptoms associated with cranial nerve dysfunction may affect persons with chronic CNS involvement. Respiratory symptoms: Cough and dyspnea develop in up to 19% of persons with brucellosis; however, these symptoms are rarely associated with active pulmonary involvement. Pleuritic chest pain may affect patients with underlying empyema.

Physical: Subclinical, acute, subacute, and chronic infections are the classic categorizations of brucellosis. Localized and relapsing forms have also been described. This classification system is subjective and has limited clinical use. Subclinical brucellosis: Disease is usually asymptomatic, and the diagnosis is usually established incidentally after serologic screening of persons at high risk of exposure. Culture data are usually unrevealing. Acute or subacute brucellosis: Disease can be mild and self-limited (eg, *B. abortus*) or fulminant with severe complications (eg, *B. melitensis*). Associated symptoms can develop at 2-3 months and 3-12 months prior to diagnosis, respectively. Chronic brucellosis: The diagnosis is typically made after symptoms have persisted for 1 year or more. Low-grade fevers and neuropsychiatric symptoms predominate. Results of serologic studies and cultures are often negative; without confirmatory evidence, many authorities doubt the existence of chronic disease. Many patients have persistent disease caused by inadequate initial therapy, and underlying localized disease may be present.

Localized complications of brucellosis are typically observed in patients with acute disease or chronic untreated infection. Osteoarticular, genitourinary, and hepatosplenic involvement are most common. Cultures of involved tissue sites and serology can be diagnostic. Relapsing brucellosis may be difficult to distinguish from reinfection. Presenting symptoms typically reflect the initial disease; however, these symptoms are more severe. Symptoms typically develop 2-3 months after therapy completion. Culture results are typically positive, and serology may be difficult to interpret, but enzyme-linked immunoassay (ELISA) testing may be more helpful. Physical findings in patients with brucellosis vary and are nonspecific for the disease. The most common findings include hepatosplenomegaly (or isolated hepatomegaly or splenomegaly) and osteoarticular involvement. Osteoarticular findings can include tenderness and swelling over affected joints, bursitis, decreased range of motion, and joint effusion (rare). Maneuvers that isolate the sacroiliac joint may cause pain.

Neurologic findings vary according to the presentation of neurologic disease, as follows: Acute meningoencephalitis (most common neurological manifestation) - Depressed level of consciousness, meningeal irritation, cranial nerve involvement, coma, seizure, and respiratory depression. Peripheral polyradiculoneuropathy - Hypotonia and areflexia in most cases, paraparesis, and an absence of sensory involvement. Diffuse CNS involvement - Spasticity, hyperreflexia, clonus, extensor plantar response, sensorineural hearing loss, cranial nerve involvement, and cerebellar signs. Cutaneous manifestations develop in 5-10% of patients, are transient and nonspecific, resolve with therapy, and do not alter the prognosis. Lesions reported in association with brucellosis are as follows: Erythema nodosum, abscesses, and papulonodular eruptions (most common), Impetigo, psoriatic, eczematous, and pityriasis rosea –like lesions, Macular, maculopapular, and scarlatiniform rashes, Vasculitic lesions (eg, petechiae, purpura, thrombophlebitis), Ocular findings can include the following: Uveitis, Keratoconjunctivitis, Iridocyclitis, Nummular keratitis, Choroiditis, Optic neuritis, Metastatic endophthalmitis, Cataracts.

Causes

Ingestion of unpasteurized goat milk and related dairy products is the main route of *B. melitensis* transmission to humans. Slaughterhouse workers, primarily those in the kill areas, become inoculated through aerosolization of fluids, contamination of skin abrasions, and splashing of mucous membranes. Farmers and shepherds have similar exposure risks, and they also have exposure to aborted animals. Veterinarians are usually infected by inadvertent inoculation of animal vaccines against *B. abortus* and *B. melitensis*. Laboratory workers (microbiologists) are exposed by processing specimens (aerosols) without special precautions.

Differential Diagnoses

Ankylosing Spondylitis and Undifferentiated Spondyloarthropathy, Influenza, Cryptococcosis, Leptospirosis, Hepatitis, Viral, Malaria, Histoplasmosis, Tuberculosis, Infectious Mononucleosis, Tuberculosis of the Genitourinary System, Infective Endocarditis, Typhoid Fever.

Other Problems to Be Considered: Collagen-vascular disease, Chronic fatigue syndrome, Malignancy, Osteomyelitis.

Workup

Laboratory Studies: As mentioned above, symptoms and signs of brucellosis are unspecific; cultures and serology are usually necessary for diagnosis. Some general laboratory findings might suggest the diagnosis eg, leukopenia, relative lymphocytosis, pancytopenia [in up to 20% of cases]. Slight elevation in liver enzymes is a very common finding. The criterion standard test for diagnosis of brucellosis is the isolation of the organism from the blood or tissues (eg, bone marrow, liver aspiration).

Culture: The sensitivity of blood cultures with improved techniques such as the Castaneda bottles is further improved by the lysis-centrifugation technique. With these methods, the sensitivity is approximately 60%. Subcultures are still advised for at least 4 weeks; thus, if brucellosis is suspected, the laboratory should be alerted to keep the cultures for 3-4

weeks, which is not done routinely for most bacterial cultures. Bone marrow culture is thought to be the criterion standard, since the reticuloendothelial system holds a high concentration of brucellae. Sensitivity is usually 80-90%. Any fluid can be cultured (eg, synovial, pleural, cerebrospinal), but the yield is usually low.

CSF evaluation: This reveals a mild-to-modest lymphocytic pleocytosis in 88-98% of in patients with neurobrucellosis. Protein levels are elevated in conjunction with normal glucose levels.

Serology

Serological testing is the most commonly used method of brucellosis diagnosis. Serum tube agglutination test: This test, developed by Bruce, measures antibodies against smooth LPS; it remains the most popular test tool for the diagnosis of brucellosis. Other tests such as tray agglutination (TAT) and modified TAT are also popular. Titers of more than 1:160 in conjunction with compatible clinical presentation is considered highly suggestive of infection. Titers of more than 1:320 are considered to be more specific, especially in endemic areas. Seroconversion and evolution of the titers can also be used for diagnosis. The shortcomings of this test include cross-reactivity with immunoglobulin M (IgM) of other organisms such as *Francisella tularensis, Salmonella urbana, Yersinia enterocolitica, Vibrio cholera, Afipia clevelandensis,* and some other bacteria. Prozone phenomenon may occur secondarily to hyperantigenemia, possibly leading to false-negative results, so routine dilution of the serum beyond 1:320 would help to prevent such a problem.

ELISA: This technique has been gaining popularity in the last few years. ELISA typically uses the cytoplasmic proteins as antigens and measures IgM, IgG, and IgA, allowing for better interpretation, especially in cases of brucellosis relapse. This is because antibodies against LPS, which are used in agglutination tests, might persist for longer periods and are believed to yield higher sensitivity and specificity. ELISA of CSF titers is also helpful in diagnosing neurobrucellosis. Because levels should decrease with effective treatment, ELISA is also helpful in follow-up.

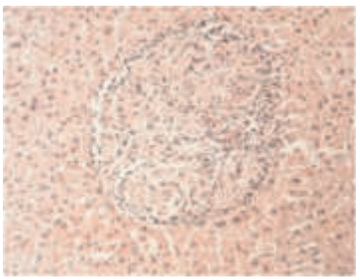
RAPID POINT-OF-CARE ASSAYS: These are available and enable fast and accessible diagnostic capabilities, especially in areas were special laboratory resources are lacking. Polymerase chain reaction (PCR): PCR testing for brucellae is a recent advance with promising potential. It would allow for rapid and accurate diagnosis of brucellosis. PCR was first developed in the early 1990s. Two major genetic targets are the Brucella gene BCSP31 and the 16S-23S rRNA operon. The 16S-23S rRNA operon has been shown in studies to be more reliable in terms of sensitivity but is not yet widely used in clinical practice and needs more standardization. Possible applications would include evaluating cases of relapse and monitoring response to therapy. Other promising tests include nested PCR, real-time PCR, and PCR-ELISA, but the clinical role for these tests remains to be defined.

Imaging Studies

Chest radiography: Radiographic findings are typically absent in brucellosis, even in patients with prominent respiratory symptoms. Findings observed in patients with active pulmonary involvement include hilar and paratracheal lymphadenopathy, pulmonary nodules, pleural thickening, and pleural effusion.

Spinal radiography: Radiographic findings in patients with osteoarticular disease occur later in the course of illness, usually 2-3 weeks after the onset of symptoms. In patients with sacroiliitis, the most commonly observed abnormalities include blurring of articular margins and widening of the sacroiliac spaces. Spondylitis-related abnormalities include anterosuperior vertebral angle epiphysitis, spinal straightening, narrowing of the intervertebral disc spaces, end-plate sclerosis, and osteophytes.

Radionuclide scintigraphy: This study is more sensitive for revealing skeletal abnormalities, especially early in the disease, when standard radiographic findings are usually normal. Radionuclide scintigraphy may be especially helpful in distinguishing hip involvement from sacroiliitis. To facilitate prompt diagnosis, this study also may have a role in screening for newly onset brucellosis and musculoskeletal symptoms.



Histologic Findings

Histologic findings in brucellosis usually include mixed inflammatory infiltrates with lymphocytic predominance and granulomas (in up to 55% of cases) with necrosis.

Well-formed hepatic granuloma from a patient with brucellosis.

Treatment

Medical Care: The goal of medical therapy in brucellosis is to control symptoms as quickly as possible to prevent complications and relapses. Multidrug antimicrobial regimens are the mainstay of therapy because of high relapse rates reported with monotherapeutic approaches. The risk of relapse is not well understood, as resistance is not a significant issue in treating brucellosis. The World Health Organization recommends the following for adults and children older than 8 years: Doxycycline 100 mg PO bid and rifampin 600-900 mg/d PO: Both drugs are to be given for 6 weeks (more convenient but probably increases the risk of relapse). Doxycycline 100 mg PO bid for 6 weeks and streptomycin 1 g/d IM daily for 2-3 weeks: This regimen is believed to be more effective, mainly in preventing relapse. Gentamicin can be used as a substitute for streptomycin and has shown equal efficacy. Ciprofloxacin-based regimens have shown equal efficacy to doxycycline-based regimens. Children younger than 8 years: The use of rifampin and trimethoprim-sulfamethoxazole (TMP-SMX) for 6 weeks is the therapy of choice. Relapse rate appears to be approximately 5% or less. Pregnant women: Brucellosis treatment is a challenging problem with limited studies. The recommendation is a regimen of rifampin alone or in combination with TMP-SMX. However, TMP-SMX use by the end of pregnancy is associated with kernicterus. In patients with spondylitis, doxycycline and rifampin combined with an aminoglycoside (gentamicin) for the initial 2-3 weeks followed by 6 weeks of rifampin and doxycycline is usually recommended. Patients with meningoencephalitis may require doxycycline in combination with rifampin, TMP-SMX, or both. A brief course of adjunctive corticosteroid therapy has been used to control the inflammatory process, but studies are limited. Patients with endocarditis require aggressive therapy. Aminoglycoside therapy in conjunction with doxycycline, rifampin, and TMP-SMX for at least 4 weeks followed by at least 2-3 active agents (without aminoglycosides) for another 8-12 weeks is preferred. Many other drugs have good *in vitro* activity against Brucella, including, but not limited to, chloramphenicol, imipenem-cilastin, and tigecycline. Gentamicin-loaded microparticles and immune-response stimulates may hold future promise. The development of an effective Brucella vaccine for use in humans would be an important step to controlling and probably eradicating brucellosis. However, the vaccine strategy is currently applicable only in control of livestock disease.

Surgical Care: The role of surgery in patients with brucellosis lies in the treatment of endocarditis or drainage of focal abscesses. Previously healthy native valves, diseased native valves, and prosthetic valvular structures have been involved in brucellosis. Valvular lesions are typically large and destructive, regardless of the organism involved.

INTERPRETATION

CD4 COUNTS, HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART) AND HIV MANAGEMENT

(Continued from previous issue)

Recovery of CD4 cells as a prognostic marker for patients on HAART

The extent of recovery of CD4 cells, once the patient has been placed on HAART, appears to be another important predictor of treatment success; patients who achieve close to normal values could potentially have a normal lifespan. Most studies focus on reducing AIDS-related mortality with progressive increases in CD4 counts while on treatment. Among previously HAART-naive patients, those who obtain CD4 counts between 500–649 cells/mm^3 had a 55% higher risk of AIDS or death compared with those with values of at least 650 cells/mm^3. However, several studies have clearly illustrated that at higher CD4 counts non-AIDS-related diseases, specifically malignancies, cardiovascular, liver and kidney disease, account for the majority of deaths. In a recent study, the risk of non-AIDS-defining deaths was reduced by approximately 30% for non-AIDS infections, end-stage liver disease and non-AIDS malignancies for each 100 cell/mm^3 increment in the latest CD4 count. Similar data has been reported in other studies. A fascinating study by Gutierrez et al. demonstrated that among patients starting HAART with sustained virologic suppression, immunologic nonresponders (defined in this study as a < 50 cells/mm^3 increase in CD4 counts after 1 year on HAART) had more than four-times greater rate of non-AIDS-related death, compared with those with satisfactory immunologic responses. All together, these findings suggest that subclinical immunodeficiency may be related with long-term risks, both AIDS and non-AIDS related. In line with this, the recent US Department of Health and Human Services guidelines recommend defining immunological failure as the lack of an increase in CD4 counts to more than 350–500 cells/mm^3 after 4–7 years of effective HAART.

Absolute CD4 count versus CD4 percentage

Several studies show the utility of the CD4 percentage in providing additional information about prognosis and when to initiate antiretroviral therapy. The CD4 percent appears to be most useful in patients with CD4 counts above 200 cells/mm^3. One cohort study by Moore et al. demonstrated that in patients starting HAART with an absolute CD4 count of 200–350 cells/mm^3, a CD4 percentage of less than 15% was associated with a markedly increased risk of mortality (relative hazard [RH]: 2.71), in comparison to those subjects with a similar baseline CD4 count but a CD4 percentage above 15%. Another retrospective cohort study by Pirzada et al. demonstrated that CD4 percentage is superior to absolute CD4 counts in predicting time to an AIDS-related event, including for patients not yet on HAART with CD4 counts between 200–350 cells/mm^3. A third study by Guiguet et al. demonstrated that CD4 percentage has additional prognostic values in terms of progression to an AIDS-defining event or death in patients with a CD4 count of 350–500 cells/mm^3; patients with an absolute CD4 count in this range but a CD4 percentage below 15%, were found to be at risk for an AIDS-defining event or death similar to that of patients with an absolute CD4 count of 200–350 cells/mm^3 but a CD4 percentage of over 15%. A study by Hulgan et al. demonstrated that patients with an absolute CD4 count of over 350 cells/mm^3 and a CD4 percent below 17% had earlier disease progression, compared with those with a CD4 percent above 17%. It is somewhat surprising that CD4 percentage adds such predictive value even in patients with absolute CD4 counts above 350 cells/mm^3. Some of the additional value of the CD4 percentage may be due to the fact that absolute CD4 counts vary depending on the time of day and other factors (e.g., in acute infection) – CD4 percentage is less subject to this variability. However, absolute CD4 count continues to be the superior prognostic indicator for patients with CD4 counts lower than 200 cells/mm^3.

Discordant CD4 count & HIV-1 viral load

Even though definitions of immunologic success vary between studies, individuals with discordant responses on HAART ('virological only', without an appropriate immunological response, or 'immunologic only' without viral suppression) consistently do worse than individuals with complete responses (both virological and immunological), yet generally do better than those with no response. One observational, multicenter study found that after 4 years of follow-up, the rate of clinical disease progression was six-times greater in nonresponders, 1.9-times greater in virologic-only responders and 2.3-times greater in immunologic-only responders. However, patients with virologic-only response or with immunologic-only response had a significantly reduced risk for clinical progression than nonresponders. Other studies have demonstrated similar outcomes, with discordant responses being significantly associated with an earlier development of an OI or death. An interesting study by Tan et al. examined a group of treatment-naive patients starting on HAART between 1995 and 2004. Among these patients, 70% experienced a complete response, 16% experienced an immunologic-only response, 9% had a virologic-only response and 5% had a concordant unfavorable response (neither viral suppression nor an increase of >50 cells/mm^3 in CD4 count). Patients who experienced discordant virologic and immunologic responses had a RH of 2.28 for the development of an OI or death, compared with those with a complete response; those in the nonresponse group had an RH of 4.83 for the development of OI or death, compared with the complete response group. Similarly, a study by Moore et al. demonstrated that a discordant immunologic and virologic response was an independent risk factor for mortality.

Adequate immunological response despite virological failure (immunologic only)

Risk factors for immunologic-only response include younger age, a lower baseline CD4 count, higher baseline viral load, poor adherence to therapy and antiretroviral drug resistance Box 2. Although the underlying mechanism is not entirely understood, less doubt exists on the management of this type of discordant response. Since current guidelines strongly emphasize the need to achieve undetectable viral loads, treatment change is usually recommended unless treatment options are limited or other underlying causes can be identified.

Poor immunological response despite effective virological response (virologic only)

Most studies have indicated that patients starting on HAART with a lower baseline CD4 count are more likely to develop a virologic-only response, probably related to disturbances in regulatory functions over T-cell homeostasis. In addition, older age also seems to be associated with a lower degree of immune reconstitution, even with successful viral suppression Box 2. Some data have demonstrated that this is related to a decreased thymic activity in older individuals. Other risk factors for an inadequate immunologic response may include the use of didanosine/ tenofovir-containing regimens. The pathophysiologic reason for failure to reconstitute a normal CD4 T-cell population despite sustained virologic suppression is an area of ongoing investigation. Several studies have found that genetic polymorphisms, including the Fas receptor (CD95) gene, the Fas ligand (CD178), the IL-6 gene, and the MHC genes are involved in T-cell immunity and affect whether an individual experiences an immunologic response to HAART therapy or not. Some investigators have asserted that the lack of reconstitution may be related to increased chronic T-cell activation, higher levels of T-cell apoptosis and a lower production of naive T cells. A recent study by Marziali et al. showed that immunologic nonresponders to HAART (defined as those who experienced <20% increase in CD4 count, or an absolute count <200 cells/mm3 following at least 1 year of therapy) differed from immunologic responders in several ways: reduced numbers of naive and thymic T cells, higher levels of IL-7

indicating persistent immune activation, lower numbers of regulatory T cells and a reduced expression of the IL-7 receptor (IL-7Rα) on CD4 and CD8 T cells. A recent, intriguing case series by Nies-Kraske et al. suggests that fibrosis of the T-cell zone of lymphoid tissue may also be an important factor in the failure to reconstitute T cells. Together, these findings demonstrate that the immune systems of immunologic nonresponders may differ from those of individuals with a successful immune response to HAART.

CD4 count & immune reconstitution inflammatory syndrome

The development of immune reconstitution inflammatory syndrome (IRIS) can occur in the first weeks to months following HAART initiation, particularly among patients with a co-existing OI. IRIS occurs as the immune system is reconstituted during the early period on HAART and is caused by a pathologic immune response to a latent or active infection, which leads to increased inflammatory symptoms. This can occur in response to any infection; however, some of the most common ones include *Mycobacterium tuberculosis*, other mycobacterial infections, herpes zoster, cytomegalovirus, Pneumocystis (carinii) jiroveci pneumonia and *Cryptococcus neoformans.*

CD4 count and CD4 percentage before starting HAART have been shown to be important risk factors in determining which patients develop IRIS after the initiation of HAART. One cohort study of an ethnically diverse population starting on HAART demonstrated that a CD4 percentage of less than 15% was associated with a greater risk of development of IRIS by nearly three-times compared with a CD4 percentage over 15% (OR: 2.97 for a CD4 percentage > 10%, and 2.59 for a CD4 percentage of 10–15%). A recent case-control study at Johns Hopkins University Hospital (MD, USA) revealed that a CD4 count of under 100 cells/mm3 was a strong independent risk factor for the development of IRIS (OR: 6.2). Other studies have shown similar associations between a lower nadir CD4 count and a higher risk of developing IRIS. The rate of the increase of CD4 count after initiation of HAART may also be a risk factor in the development of IRIS, with patients who have a more rapid rise in CD4 count being at a higher risk for developing IRIS; however, not all studies have confirmed this.

(To be continued....)

BOUQUET

In Lighter Vein

Medical Terminology Twisted

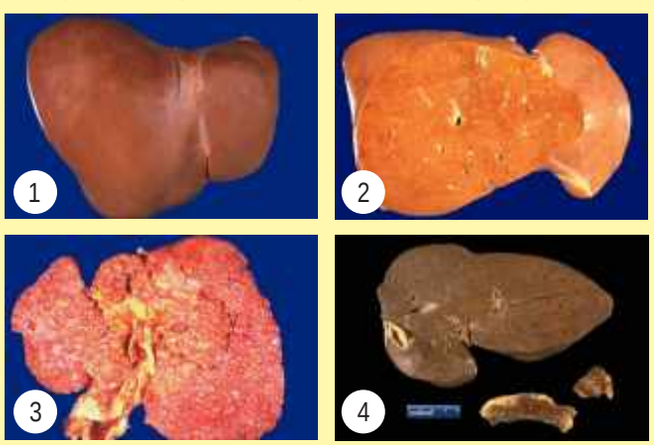
Artery	—	Study of paintings
Bacteria	—	Back door of cafeteria
Barium	—	What doctors do when treatment fails
Bowel	—	Letter like A.E.I.O.U
Caesarean section	—	District in Rome
Cat scan	—	Searching for kitty
Cauterize	—	Made eye contact with her
Colic	—	Sheep dog
Coma	—	A punctuation mark
Congenital	—	Friendly
D&C	—	Where Washington is
Diarrhea	—	Journal of daily events
Dilate	—	To live long
Enema	—	Not a friend
Fester	—	Quicker
Fibula	—	A small lie
G.I. Series	—	Soldiers' ball game
Grippe	—	Suitcase
Hangnail	—	Coathook
Impotent	—	Distinguished, well known
Intense pain	—	Torture in a teepee
Labor pain	—	Got hurt at work
Medical staff	—	Doctor's cane
Morbid	—	Higher offer
Nitrate	—	Cheaper than day rate
Node	—	Was aware of
Outpatient	—	Person who had fainted
Pelvis	—	Cousin of Elvis
Post operative	—	Letter carrier
Protein	—	Favoring young people
Rectum	—	It almost killed him
Recovery room	—	Place to do upholstery
Rheumatic	—	Amorous
Scar	—	Rolled tobacco leaf
Secretion	—	Hiding anything
Seizure	—	Roman emperor
Serology	—	Study of knighthood
Tablet	—	Small table
Terminal illness	—	Sickness at airport
Tibia	—	Country in North Africa
Tumor	—	An extra pair
Urine	—	Opposite of you're out
Varicose	—	Located nearby
Vein	—	Conceited

Wisdom Whispers

- "Despise learning and make everyone pay for your ignorance."
- "There is misfortune only where there is wealth."
- "Variety is the spice of life."
- "No fine clothes can hide the clown."
- "Fear is a fine spur; so is rage."
- "Do as you would be done by."
- "A miser is ever in want."
- "Every cook praises his own broth."
- "Assertion is no proof."
- "'Tis a silly sheep that makes the wolf her confessor."
- "The most covered fire is always the most glowing."
- "Do not buy a carrier's ass, or marry an innkeeper's daughter."
- "Hide not your light under a bushel."
- "You have to strike while the iron is hot."

Brain Teasers

Identify the following and pathologies of liver as seen grossly



Answers: Fig.1. Normal liver, 2. Fatty change liver, gross picture, 3. Macronodular cirrhosis of liver, 4. Haemochromatosis of liver.

TROUBLESHOOTING

QUALITY ASSURANCE IN URINE TESTING

QUALITY ASSURANCE (Q.A.): It is a program of checks and balances to assure the quality of laboratory services. It is a mechanism to detect problems and improve services that includes plans, policies, and procedures. Q. A. encompasses every ancillary service of the institution. The following components are part of the Q. A. process in the laboratory; (1) how tests are ordered, (2) the quality of the request forms, (3) identification of patients, (4) how specimens are procured, (5) how specimens are labeled, (6) how specimens are transported to the lab, (7) how specimens are processed, (8) how tests are performed and if there is an established procedure for each test, (9) laboratory procedure manuals for every department, (10) laboratory instruments, (11) quality of reagents, (12) turnaround time, (13) accuracy of results that are reported, (14) how is quality control in the laboratory conducted, (15) procedures for going about to solve problems, (16) how are patient's records kept, (17) are critical values established, and (18) what proficiency test procedures are in place to assure accuracy of results. Q. A. is a process that included the interaction between the patient, the laboratory, other departments, and the physicians. Included in this process is included evaluation, monitoring, documentation, and communication to remove obstacles to appropriate patient care. Its intent is to give self confidence to the laboratory staff performing the tests.

QUALITY CONTROL (Q. C.) IN THE LABORATORY: Q. C. is a system that ensures reliable test results in the clinical laboratory. This system monitors the facilities, test methods, test equipment, reagents, supplies, procedures, equipment maintenance, calibration strategies, control procedures, remedial actions, and maintains a record book.

RELIABILITY, ACCURACY, PRECISION, AND VARIANCE ARE PART OF THE QUALITY CONTROL PROGRAM: Reliability is dependent upon the accuracy and precision of test results. An accurately performed test describes how close the test results are to the actual value of the test. A test that can be repeated over and over, still obtaining the same results describes the obtained that are statistically close to the actual value and when repeated and the same results are obtained, then the test is reliable. Accuracy and precision of a test depends upon the laboratory using standardized procedures, control standards, reputable reagents and supplies, and precise equipment. Variance is a statistical term that describes how much fluctuation occurs when a specimen is tested repeatedly. The smaller the fluctuation the smaller the variance and the more reliable the test.

CONTROL SPECIMENS AND THE Q. C. PROGRAM: A control specimen is an sample specimen that resembles the patient's test specimen, but the constituents are known. Generally control specimens consists of low abnormal, normal, and high abnormal values. The control specimens are entered into the testing procedure and treated as a patient specimen. In this way, by knowing the expected outcome of a control specimen, any variable that would affect the patient specimen will also affect the control. Through the control specimen; the parameters of reliability, precision, accuracy, and variance are monitored and the results of the testing procedure assured.

SEVEN FACTORS THAT MAY CAUSE A TEST RESULT TO BE UNACCEPTABLE: (1) The reagents or controls have deteriorated. (2) Defect in equipment/ instrument. (3) Glassware improperly cleaned or is contaminated. (4) Failure in correctly timing the incubation temperatures. (5) Failure to use a method appropriate for the equipment/instruments. (6) Carelessness/ indifference by the laboratory staff performing the test. (7) Murphy's law. If something can go wrong it will and you won't know why. Statistically for some unknown reason a test result will be in error.

Q. C. AS PART OF SPECIMEN EVALUATION FOR ACCEPTABILITY OF A URINE SPECIMEN FOR TESTING: Specimens must be received into the laboratory that are suitable for testing. The laboratorian must be able to recognize whether or not a specimen will be able to provide the kind of results that a physician can use in the care of a patient. The following seven points address this process. (1) Incorrect preservative used in the urine. If a urine is preserved with formalin and a leukocyte esterase test is requested, the results will be a false positive. (2) An insufficient volume of urine for the requested test. If procedure calls for 1.0 mL of urine and the lab receives 0.5 mL, another specimen should be collected. (3) The specimen is not labeled. It is impossible for the laboratory to guarantee that the results are for a specific patient. (4) The specimen or requisition form is improperly labeled. Same reason as #3. (5) The specimen is inappropriate for the test requested. If a random specimen is brought in for a culture and sensitivity in an unclean container, the results would be invalid and unnecessary costs to the Patient. (6) Visibly contaminated urine. If the urine contains fecal material, it is invalid for tests. (7) Specimen not collected properly for transportation to a reference lab. The preservative may not be correct or the amount in container is wrong. The lab cannot guaranteed reliable results.

TEN ERRORS THAT CAN OCCUR IN URINE TESTING IF A LABORATORY FAILS TO HOLD TO Q. A. PRINCIPLES: (1) Inadequate care of reagents (failure to replace bottle cap or improper temperature storage). (2) Testing urine that is not fresh (usually more than 1-2 hours old). (3) Failure to mix urine before testing (this is the most common error in the lab). (4) Failure to follow directions, tends to use short cuts, poor technique. (5) Not able to recognize false positive or false negative results. The laboratorian does not understand what substances can interfere with a test. (6) Using chemically unclean containers. There may be residual cleaning materials present. (7) Failure to recognize critical values or when values are inconsistent with each other. (8) Failure to recognize when a test must be repeated. The tech has a disregard for test results. (9) Recording wrong results. 2.22 mg/dL is different from 22.2 mg/dL. (10) Lack of understanding of the importance of incorporating test data from several tests to make a clinical diagnosis. The laboratorian can use such data to verify his/her results.

LIST OF EQUIPMENT ITEMS AND THE FREQUENCY AT WHICH PERFORMANCE EVALUATION MUST BE DONE: (1) Thermometers: Test when received into the department and test once annually using a National Bureau of Standards certified thermometer. (2) Temperature devices (refrigerators, dry-block baths, etc.): Test daily by recording the temperature and recording in a log. (3) Reagent strip readers: Test daily by using standard reagent strips and control urine. (4) Microscopes: Perform daily. Perform maintenance as prescribed in the procedure manual. (5) Centrifuges: Clean daily with disinfectant. Check r.p.m. with a tachometer and timer with a stop watch on an annual basis. (6) Automatic pipettes: Test when received into the laboratory and then annually per the laboratory procedure manual.

HOW TO CORRECTLY HANDLE MISLABELED OR NON-LABELED URINE SPECIMENS: (1) If unlabeled or mis-labeled, set the specimen and its requisition aside in a container. (2) Obtain an "incident sheet" and record responses and actions. (3) Notify the collection source (nursing station, clinic, etc.). State the problem and request that a new specimen be collected. Record the name of the person talked to, date, time, and any other data. (4) Do not discard the specimen. Retain until the matter is resolved and determined to be completed. (5) If the specimen cannot be re-collected, then one of the following must occur: (a) The physician must contact the laboratory and request testing on the questionable sample. He must also signature the incident document. (b) The person responsible for collecting the specimen must come to the laboratory and identify the specimen, properly label the specimen and/or requisition form. They must also sign off on the incident form. (6) Perform the test and report results after signatures are secured. (7) Dependent upon the circumstances, the report may need to contain the following comment, "This specimen was improperly labeled (or whatever the problem), but was approved for testing. The laboratory cannot otherwise guarantee that the reported values are from this patient. (8) Forward a copy of the incident report to the Q. A. committee and the unit which sent the specimen.

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