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The Crux

BIMONTHLY FORUM FOR THE LABORATARIANS

CONTENTS

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Editorial

On receipt of several requests to cover Leprosy, we have done so in this issue and laid it threadbare for you. Henceforth, you should have no hiccups while dealing with cases of Hansen's disease. All relevant clinico-diagnostic aspects are entertained. A detailed classification correlating clinical picture with diagnostic findings has been considered in ample depth. Leprosy even till date occupies an important platform in developing countries as far as communicable diseases are concerned. Though absolutely curable if diagnosed early, the deforming complications too can be prevented.Newer diagnostic tools like PCR here too come to the rescue of a diagnostician. A laboratarian has a tremendous role to play (though this role mostly goes unsung, all the credit goes to the person who writes the therapeutic regimen).

INTERPRETATION segment interprets hyperbilirubinemia and urinary/ fecal urobilinogen for you. A detailed aetiological classification is provided. This would definitely assist in identifying the cause of jaundice when read in conjunction with reports of other parameters.

All the processes conducted in a diagnostic medical laboratory commence with the specimen preparation. The TROUBLE SHOOTING section profusely outlines all problems that can be encountered while handling clinical samples. It tells you how to prepare all clinical samples before you start the diagnostic exercises. They say first things first. The first thing in our clinical practice is the sample preparation.

BOUQUET is there as usual. We do not laugh at others, we laugh at our usual behaviours and ourselves. A little advise from the great people who said great things. And lastly few haematological questions to tickle your brain under Brain Teasers.

Happy Reading! Any topics of interest to you in particular, we'll be more than pleased to cover them in our forthcoming issues.

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DISEASE DIAGNOSIS

LEPROSY (HANSEN'S DISEASE)

Description

Leprosy is a chronic granulomatous infectious disease caused by the acid-fast bacillus *Mycobacterium leprae*. **The** disease is characterized by disfiguring skin sores, peripheral nerve damage, and progressive debilitation, which has led to victims being ostracized from their communities and their families. **Leprosy** was recognized in the ancient civilizations of China, Egypt, and India, with the first known written reference dated 600 BC. The incidence of the disease peaked in the 13th century. **In** 1873, the Norwegian Gerhard Henrik Armauer Hansen discovered *M. leprae*, making it the first bacillus, and second bacterium, to be associated with a human disease.

Synonyms

Hansen's disease, Paucibacillary leprosy, Multibacillary leprosy, Indeterminate leprosy, Tuberculoid leprosy, Borderline tuberculoid leprosy, Borderline leprosy, Borderline lepromatous leprosy, Lepromatous leprosy, Lepra reaction, Neuritic leprosy

Background

Cardinal features: The cardinal features of leprosy are: **Skin** patch with sensory loss. **Nerve** enlargement. **Acid-fast** bacilli in the skin. Of these, the most important is nerve enlargement, as 30% of patients, including many lepromatous leprosy patients, may not present with a skin patch with sensory loss, and some patients simply have leprosy as a neural disease, known as neuritic leprosy. **The** manifestations of leprosy are determined by the host's immunopathologic responses to the organism, resulting in a wide clinical spectrum that was characterized by Ridley and Jopling in 1966 as five distinct forms. **The** polar forms of leprosy - tuberculoid (TT) and lepromatous leprosy (LL) - are immunologically stable, while the intermediate types of borderline tuberculoid (BT), borderline (BB), and borderline lepromatous (BL) are immunologically unstable and lead to either a gradual decline towards the lepromatous pole or upgrading 'reversal reactions' towards the tuberculoid pole. **TT** is characterized by a vigorous cellular and limited humoral immune responses to *Mycobacterium lepra*e, usually involving the skin and nerves, and resulting in few skin lesions. LL, on the other hand, is characterized by a minimal cellular and a vigorous humoral immune response and, consequently, extensive skin involvement. **Indeterminate** leprosy, a further form of leprosy, is an early form of the disease with only a small number skin lesions and no nerve involvement. **Leprosy** may also be divided into paucibacillary and multibacillary forms, depending on the number of bacilli present in skin lesions. Paucibacillary leprosy, in which there are a low number of bacilli, is equivalent to indeterminate leprosy, TT, and BT, while multibacillary leprosy approximates to BB, BL, and LL. This definition has a significant bearing on the choice of therapy. **The** dynamic immunopathological host response to *M. leprae* during the course of leprosy may result in spontaneous lepra reactions. These are more common in borderline forms of the disease and may be precipitated by treatment. Systemic corticosteroids form the mainstay of treatment for lepra reactions. See Complications for more details. **Lepra** type 1 reactions involve either a downgrading or upgrading reaction, with patients developing inflammation, neuritis, and, potentially, fever. They are a major cause of nerve damage. **Lepra** type 2 reactions, or erythema nodosum leprosum (ENL), affect around half of all patients and consist of an immune complex-mediated reaction to *M. leprae* that has widespread, systemic effects, in addition to causing the development of new skin lesions

Causes

Common causes: Leprosy is caused by chronic infection with *M. leprae*, an acid-fast, Gram-positive bacillus that is an obligate intracellular parasite with tropism for macrophages and Schwann cells. **The** incubation period is very slow, ranging from 6 months to >40 years, with an average period of 2-5 years. The slow incubation is due to a very slow dividing time of once every 2 weeks. ***M. leprae*** cannot be cultivated in vitro, but is capable of limited multiplication in the mouse footpad, with a doubling time of 12-13 days. This has permitted the conducting of drug sensitivity studies. **Genomic** studies have shown that many of the functional genes for energy metabolism in *M. leprae* are absent, having been replaced by inactivated or pseudo genes. This has resulted in the removal of entire metabolic pathways and regulatory genes, and may make the bacillus dependent on host metabolic pathways. This may explain the long generation time and inability to grow in culture. **The** bacterium is transmitted via droplets, from the nose and mouth, during frequent and close contact with untreated patients. It is estimated that approx. 50% of patients have a history of intimate contact with an infected person, usually a household member. **Although** leprosy is not highly infectious, untreated LL patients harbor large number of *M. leprae* in their nasal mucosa and secretions

Contributory or predisposing factors

Although the majority of leprosy cases are sporadic, the greatest risk factor for leprosy is household contact with untreated patients. The relative risk for household contacts of lepromatous patients is 8- to 10-fold higher, and 2- to 4-fold higher for contacts of TL patients. **Animal** reservoirs of leprosy have been found in 9-banded armadillos (of which 15% are thought be to infected in the wild in Louisiana and Texas), chimpanzees, and mangabey monkeys. **In** addition, exposure to insect vectors and infected soil have been suggested as possible modes of transmission

Epidemiology

Incidence In 2004, around 410,000 new cases of leprosy were detected worldwide, compared with a peak of 804,000 in 1998, and there has been a 20% annual fall in incidence since 2001. **However**, the case detection rate rose over the past decade, appearing to stabilize at around 700,000 new cases per year. This may be due to improved leprosy control and case finding, rather than an actual increase in leprosy incidence

Prevalence In 1985, there were an estimated 12 million people living with leprosy worldwide, giving a prevalence of 120 per 100,000 population. There were 597,000 registered cases and 719,000 new cases detected during 2000, giving a global prevalence of just below 10 per 100,000 population

Demographics

Age: Leprosy has a bimodal distribution, with peaks observed at ages 10-14 years and 35-44 years. **Children** seem to be more susceptible to developing leprosy than adults, and tend to have the tuberculoid, rather than lepromatous, form. **While** leprosy is rare in infants, those born to mothers with the disease have slow growth and decreased birth weight. Infants have an increased risk of contracting the disease if their mother has LL

Gender: In adults, leprosy is more common in men than women at a male: female ratio of 1.5-2.0:1. **There** is no difference in gender in leprosy distribution in children

Race: While leprosy occurs in all races, Africans have a higher incidence of the tuberculoid form of the disease than other groups, while the lepromatous form is more common in light skinned and Chinese individuals.

Geography: A total of 15 countries worldwide have a prevalence of leprosy >10 per 100,000 population, which is the target level set by the World Health Organization (WHO) for elimination of the disease. These countries are mainly located in the tropics and subtropics. **However**, it is noteworthy that 122 of the countries that were classified as endemic for leprosy in 1985, 107 have reached the elimination target. **Cases** of leprosy are highly concentrated, with 83% of patients located in only six countries: India, Brazil, Myanmar (Burma), Indonesia, Madagascar, and Nepal. India alone accounts for 64% of all leprosy cases worldwide. **Leprosy** has virtually disappeared from Europe, and the vast majority of cases in North America are among migrants from endemic areas. However, cases of leprosy among patients with severe immune suppression (such as organ transplant recipients) have been reported.

Socioeconomic status: Leprosy is associated with poverty and overcrowding, with improved socioeconomic conditions being a major contributor to the decline of the disease. **The** disease is also more common in rural, rather than urban, areas

Diagnosis

Clinical presentation: The classic clinical presentation of leprosy is inflamed skin lesions and/or sensorimotor neuropathy, with nerve enlargement in patients with tuberculoid leprosy. However, it is important to note symptoms and signs may take decades to appear due to the long incubation period of *Mycobacterium leprae*. Furthermore, patients have different clinical presentations depending on the form of leprosy.

Symptoms: The typical symptoms of leprosy are as follows: **One** or more hypopigmented skin lesions that have decreased sensation to touch, heat, or pain. **Skin** lesions that do not heal after several weeks or months. **Numbness** or absent sensation in the hands and arms, or feet and legs. **Muscle** weakness due to nerve damage, resulting in, for example, foot drop, in which damage to the peroneal nerve causes the toe to drag when the foot is lifted to take a step

Prodromal symptoms are often so minor that the disease is not recognized until a cutaneous eruption occurs. Approx. 90% of patients present with numbness first, potentially years before the appearance of lesions.

The first sensation to be lost is temperature, with patients unable to sense extremes of hot or cold. This is followed by light touch, then pain, and, finally, deep pressure, with the losses especially apparent in the hands and feet. People with longterm leprosy may lose the use of their hands or feet due to repeated injury resulting from lack of sensation.

Signs: The signs of leprosy vary widely, depending on the patient's form of leprosy: **Indeterminate** leprosy: in this early stage, there are one to a few hypopigmented, or sometimes erythematous, macules. Sensory loss is unusual. **Tuberculoid** leprosy (TT): skin lesions are few in number, with, usually, one erythematous large plaque with well-defined borders that are elevated and slope down to an atrophic center. The lesions can become arciform or annular, and can be found on the face, limbs, or areas other than the scalp and intertriginous areas. Patients may also have a large, asymmetric hypopigmented macule, with lesional hypesthesia and involving local alopecia. Neural involvement is common in TT, causing tender, thickened nerves with loss of function. The greater auricular nerve and superficial peroneal nerves are most commonly affected. **Borderline** tuberculoid leprosy (BT): lesions in this form of leprosy are similar to those seen in TT, but are smaller and more numerous. The nerves are also less enlarged and alopecia is less common and pronounced. **Borderline** leprosy (BB): in this stage, cutaneous lesions are numerous, red, irregular plaques that are less well defined than those seen TT. The distribution may be the same in lepromatous leprosy, but the lesions are more asymmetric. Hypesthesia is only moderate in this form, and regional adenopathy may be present **Borderline** lepromatous leprosy (BL): lesions are numerous in BL and consist of macules, papules, plaques, and nodules, with annular punched-out lesions that have the appearance of inverted saucers being common. Hypesthesia is often absent. **Lepromatous** leprosy (LL): early cutaneous lesions in LL may consist mainly of pale macules, while, later on, infiltrations are present, with numerous bacilli. The macular lesions are small, diffuse, and symmetric, and there is little or no loss of sensation and no change in skin texture. Nerves are also not thickened, and sweating is normal. Alopecia affects the lateral eyebrows (known as madarosis), which spreads to the eyelashes, and then the trunk. The scalp hair remains intact.

Further signs in LL include Lepromatous infiltrations, which can be diffuse, or in the form of nodules or plaques. Diffuse infiltration results in leonine (lion-like) facies (heavily furrowed, thickened skin folds on the forehead with prominent supraorbital ridges and loss of eye brows). Neuritic lesions are symmetric and slow to develop. **Eye** involvement. This may occur in LL and causes pain, photophobia, decreased visual acuity, glaucoma, and blindness. **Testicular** involvement, which may result in sterility and gynecomastia. **Lymphadenopathy** and hepatomegaly, which may result from organ infiltration. **Stridor** and hoarseness, which are the result of laryngeal involvement, while nasal infiltration can cause saddle-nose deformity. **Aseptic necrosis** and osteomyelitis, resulting from repeated trauma after joint invasion. **Brawny** edema of the lower extremities. This is a late finding in LL

Differential diagnosis

Other skin diseases may be differentiated from TT by the absence of lesional hypesthesia, and the presence of nerve involvement elsewhere. In contrast, LL lesions are not hypesthetic, and biopsy may be necessary in order to distinguish these from lesions due to other systemic infections, such as leishmaniasis and secondary syphilis, as well as other nodular or infiltrative skin conditions. Other causes of nerve enlargement, such as primary amyloidosis and familial polyneuropathy are excluded by biopsy and family history.

Cryptococcosis

Cryptococcosis is a systemic infection caused by the yeast-like fungus *Cryptococcus neoformans*. It is an AIDS-defining illness, and is the most common invasive fungal infection in patients with AIDS.

Features: The infection is acquired via inhalation of airborne yeast cells, and person-to- person transmission does not occur. **It** is most common in patients with HIV/AIDS and other causes of impaired cellular immunity; symptomatic cryptococcosis is uncommon in immunocompetent individuals. **It** can manifest anywhere in the body (notably skin, lungs, prostate, and eyes), but the infection has a predilection for the brain and meninges

Blastomycosis

Blastomycosis is a systemic pyogranulomatous disease caused by the fungus *Blastomyces dermatitidis*.

Features: Primary disease usually manifests as acute or chronic pneumonia. **Acute** disease is characterized by abrupt onset of myalgias, arthralgias, chills, fever, and cough (productive or nonproductive); chest X-ray usually shows lobar or segmental consolidation. **Chronic** disease is characterized by progressive disease with indolent onset. Symptoms include productive cough, hemoptysis, weight loss, pleuritic chest pain, and low-grade or no fever; chest X-ray may show mass lesions or alveolar infiltrates (lobar or segmental), sometimes with cavitation. **May** disseminate hematogenously, most often to skin, bone, or the male genitourinary system.

Sporotrichosis

Sporotrichosis is caused by a fungus that lives in soil and decaying vegetation. The infection is usually acquired through a splinter, cut, or puncture wound.

Features: Caused by fungi of the genus *Sporothrix*. **Gardeners** and greenhouse workers are at increased risk. **Initial** symptom is a pink, red, or purple nodule, which later breaks down and discharges mucoid pus. **Secondary** lesions emerge along superficial lymphatic channels. **Usually** afebrile; indolent infection involving a single extremity, frequently an upper extremity.

Nocardiosis

Nocardiosis is caused by *Nocardia spp*., which are filamentous bacteria that may cause disseminated disease in immunocompromised patients. Infection may involve the lung, brain, soft tissues, lymph nodes, and a variety of other tissues.

Features: In most immunocompetent patients, *Nocardia* infection presents as a subacute soft tissue infection from a soil-contaminated wound (*Nocardia* spp. are common inhabitants of soils in temperate climates). **More** severe infection occurs in immunocompromised patients, with inhalation of the organism followed by pulmonary infection and dissemination to extra-pulmonary sites. **An** initial pulmonary infection can be followed by progressive systemic dissemination with abscess formation affecting the central nervous system, soft tissues, liver, spleen, and lymph nodes. **Infection** by this organism is characterized by typical Gram stain appearance with irregular branching, small, poorly staining Gram-positive rods that are partially acid fast on appropriate stains. **The** organism can be cultured from a variety of culture media but are slow growing aerobic organisms that may take several weeks to identify in the clinical laboratory. **Treatment** with sulfa drugs (sulfamethoxazole-trimethoprim), minocycline, amikacin, cefotaxime, and a number of other antibiotics. Empiric treatment with sulfa drugs is often utilized to cover the possibility of toxoplasmosis and/or nocardiosis in severely immunocompromised patients while awaiting histologic or culture documentation.

Leishmaniasis

Leishmaniasis is divided into visceral, cutaneous, and mucosal syndromes. Caused by protozoa of the genus *Leishmania* and transmitted by sandflies to humans from small animal reservoirs (rodents, dogs).

Features: Visceral: recurrent fever, weight loss, hepatosplenomegaly,

anemia, leukopenia, and hypergammaglobulinemia. Visceral leishmaniasis is an important opportunistic infection in those infected with HIV. **Cutaneous:** variety of skin lesions including small, dry, crusted lesions to large, deep, mutilating ulcers. **Mucosal:** nasal stuffiness, discomfort, discharge, or epistaxis. may progress to destruction of nasal septum.

Amyloidosis

Patients with amyloidosis have skin thickening on the fingers and hands and peripheral neuropathy, which may be mistaken for advanced tuberculoid leprosy.

Features: Skin thickening on fingers and hands. Nephrotic syndrome. Hepatomegaly, Carpal tunnel syndrome, Macroglossia, Malabsorption, Unexplained diarrhea or constipation, Peripheral neuropathy, Cardiomyopathy.

Neurofibromatosis

Neurofibromatosis is an autosomal dominant condition with two distinct clinical types: type 1 and type 2.

Features: Axillary and inguinal-area freckling. **Occasional** development of peripheral sarcomas, **May** have overgrowth of subcutaneous tissues. **Café-au-lait** cutaneous pigmentation with neoplastic nodules in skin, CNS, other internal organs.

Sarcoidosis

Sarcoidosis is a multisystem granulomatous disorder of unclear etiology that is characterized by the presence of noncaseating granulomas. It can mimic several other diseases.

Features: Most commonly presents as pulmonary syndrome with hilar adenopathy, alveolitis, interstitial pneumonitis, and granulomas on histologic examination. **Systemic** symptoms are less common and include fever, weight loss, anorexia, and fatigue. **One**-quarter of patients have skin involvement with erythema nodosum, plaques, maculopapular eruptions, and subcutaneous nodules. **Uveitis** can occur. **Liver** involvement is common, but evident only on histologic examination. **Cranial** or peripheral neuropathy, chronic meningitis, and seizures may occur

Secondary syphilis

Syphilis is a sexually transmitted disease caused by *Treponema pallidum*. Secondary syphilis has a wide range of systemic symptoms.

Features: Fever. Malaise. Rash. Mucocutaneous lesions. Adenopathy. Anorexia and weight loss

Psoriasis

Psoriasis is a common autoimmune condition that is characterized by inflammation of the skin, with frequent episodes of pruritus, redness, and thick, dry, silvery scales

Features: Lesions are commonly found on the trunk, elbows, knees, scalp, skin folds, or fingernails, but can occur anywhere. **The** disease may be aggravated by injury or irritation; it is severe in immunocompromised patients and those with other autoimmune disorders, such as rheumatoid arthritis. **Medications**, infections, excessive alcohol consumption, obesity, a lack of sunlight, sunburn, stress, general poor health, and frequent friction are all linked to flare-ups

Vitiligo

Vitiligo is a skin condition in which a loss of pigment from the skin results in irregular white patches, although the skin texture is normal.

Features: Lesions are flat depigmented areas with a dark, sharply defined but irregular border. **Frequently** affects the face, elbows, knees, hands, feet, and genitalia. **It** may appear at any age and is associated with a family history of the condition

Contact dermatitis

Contact dermatitis is an inflammatory skin disorder caused by contact with irritants or allergens, including poison ivy and other plants, nickel, topical medications, rubber, cosmetics, fabrics and clothing, detergents, and solvents.

Features: May not occur on first contact with an irritant or allergen, but only after repeated contact, such as to nail polish remover, the preservatives in contact lens solution, or the back of a metal watch. **Patients** experience pruritus in the exposed area, along with redness or inflammation of the skin, tenderness, and localized swelling. **Lesions** or rashes at the site of exposure may have papules, vesicles, and bullae, and involve oozing or crusting

Workup

Diagnostic decision

According to the WHO, the diagnosis of leprosy does not require any testing. An individual should be regarded as having leprosy if he or she shows EITHER skin lesions consistent with leprosy and with definite sensory loss, with or without thickened nerves OR positive skin smears. It must also be noted that many advanced microbiologic techniques may not be available in the remote regions of the countries where the disease is endemic. Therefore, the diagnosis of leprosy is fairly straightforward, if it is suspected as a cause of any skin or peripheral nerve lesions in a person from a country in which leprosy is endemic. The cardinal features of leprosy are: **Skin** patch with sensory loss. **Nerve** enlargement. **Acid**-fast bacilli in the skin.

The presence of one or more of these features establishes the diagnosis, which should be confirmed with a full-thickness biopsy. Around 70% of leprosy patients can be diagnosed with just the sign of a skin patch with sensory loss. However, 30% of patients, including many of those with LL, may not present with this sign, which underlines the importance of nerve enlargement as an additional sign and the importance of clinical suspicion.

In order to maximize the accuracy of a diagnosis based on signs, physicians should look for a thickened nerve and one of the following: **Typical** hypopigmented or erythematous skin, with or without sensory loss. **Typical** nerve function impairment, such as the loss of sensation on the palms or soles

Leprosy may also present as purely a neural disease, without skin lesions, which is known as neuritic leprosy. In such patients, nerve biopsy is confirmatory. The proportion of leprosy patients with the neuritic form of the disease is 0.5% in Ethiopia, 4.6% in India, and 8.7% in Nepal.

Examination

Patients with a history of living or traveling in areas endemic for leprosy who present with hypopigmented or erythematous macules should undergo a thorough examination, focusing specifically on the distribution of any macules and the presence of enlarged, tender nerves. Skin lesions in leprosy are present in the cooler areas of the body, and are not found in the scalp, groin, or axilla. **A** careful sensory and motor examination should also be conducted (including tactile and temperature sensations), with a wisp of cotton used for testing for lesional hypesthesia. The first sensation to be lost is temperature, with patients unable to sense extremes of hot or cold. This is followed by light touch, pain, and, finally, deep pressure. Losses are particularly apparent in the hands and feet

Summary of tests

The presence of acid-fast bacilli in the skin is best demonstrated by slit-skin smears, which should be taken from the edges of at least two lesions and both ear lobes. If this is not possible, a skin biopsy should be stained for acid-fast bacilli using a modified Wade-Fite stain. The extent of bacillary load may be quantified as a bacterial index on a logarithmic scale of 1+ to 6+. The morphological index (MI) may also be calculated, which is the number of viable bacilli per 100 bacilli detected **In** patients with neuritic leprosy, a nerve biopsy from a sensory nerve such as the superficial radial nerve may be diagnostic. The lepromin skin test does not confirm the diagnosis, but is useful in distinguishing lepromatous leprosy (LL) from tuberculoid leprosy, while histamine testing allows the detection of postganglionic nerve damage. **Polymerase** chain reaction (PCR) testing for *Mycobacterium leprae* DNA and antibody tests for antibodies to *M. leprae*-specific phenolic glycolipid (PGL)-1 and other *M. leprae*-specific proteins present in LL patients may also be conducted. However, these are expensive and more commonly confined to epidemiologic studies. **Histologic** findings vary but can include dermatitis, giant cells, infiltration of nerve bundles with mononuclear cells, and granulomas. Lepromatous lesions typically contain numerous acid-fast bacilli and fat-laden macrophages, with a paucity of lymphocytes. In contrast, tuberculoid lesions contain few, if any, acid-fast bacilli, but reveal granulomatous changes

INTERPRETATION

SERUM BILIRUBIN/ URINARY AND FECAL UROBILINOGEN

Normal general reference range: *Total bilirubin upto 1.0 mg/dL*
Conjugated bilirubin upto 0.25 mg/dL

Causes of Hyperbilirubinemia

UNCONJUGATED (INDIRECT) HYPERBILIRUBINEMIA

I Overproduction of bilirubin
- A. Hemolytic disorders.
  1. Congenital (e.g.,hemoglobinopathies).
  2. Acquired (e.g., Coomb's positive anemia)
  3. Liver disease (e.g., Hepatitis and cirrhosis).
- B. Shunt hyperbilirubinemia

II. Defective uptake and storage of bilirubin
- A. Idiopathic unconjugated hyperbilirubinemia
  1. Hereditary Gilbert's syndrome
  2. Acquired
     Post-viral hepatitis.
     Post-portacaval shunt
- B. Decreased availability of cytoplasmic binding proteins (Y and Z) in newborn and premature infants.
- C Drugs (e.g., flavispidic acid)

III. Defective Glucuronyl Transferase activity.
- A. Deficiency.
  1. In newborn and premature infants
  2. Crigler-Najjar syndrome.
- B Inhibition
  1. Abnormal steroids in breast milk or maternal plasma (Lucey-Driscoll type)
  2. Drugs (e.g., Novobiocin).

CONJUGATED (DIRECT) HYPERBILIRUBINEMIA Defective excretion of conjugated bilirubin
- A. Hereditary
  1. Dubin-Johnson syndrome.
  2. Rotor syndrome.
- B. Obstructive
  1. Intrahepatic cholestasis.
     (a) Cirrhosis (occasionally)
     (b) Hepatitis(often)
     (c) Alcoholic liver disease(occasionally)
     (d) Drugs(e.g.,chlorpromazine and methyl testosterone)
     (e) Primary biliary cirrhosis
  2. Extrahepatic obstruction.
     (a) Gall stones
     (b) Carcinoma of the bile duct pancreas, ampulla of Vater
     (c) Bile duct stricture.
     (d) Biliary atresia.

UNCONJUGATED BILIRUBIN
- A. Pre-hepatic (hemolytic retention jaundice)
  1. Excessive red cell hemolysis
     (a) Familial: e.g., spherocytosis, enzyme defects in red cell
     (b) Acquired:
         Traumatic, e.g., Hematomas
         Toxic, e.g. Phenylhydrazine
         Infective, e.g., Malaria
         Neoplastic, e.g., Hodgkin's disease.
  2. Excessive "shunt" production.
- B Hepatic

Nonhemolytic retention jaundice (defect of transport into cell or microsomes)
  (a) Familial: UDP glucuronyl transferase deficiency (Types I and II), Gilbert's disease, Crigler-Najjar syndrome
  (b) Acquired or uncertain inheritance: Neonatal jaundice, e.g., Physiological breast milk, or serum factor.

CONJUGATED BILIRUBIN

Intrahepatic cholestasis (regurgitation jaundice).
- A. Hepatocellular injury:
  1. Toxic, e.g., carbon tetrachloride necrosis
  2. Infective, e.g., Viral hepatitis
  3. Neoplastic, e.g., primary or secondary carcinoma of liver
  4. Cirrhosis, e.g., familial or acquired.
- B. Bile duct injury:
  1. Familial, e.g., Dubin-Johnson syndrome. Rotor syndrome, recurrent familial cholestasis
  2. Toxic, e.g., drugs (phenothiazines, steroids).
  3. Inflammatory, e.g., sclerosing cholangitis.
  4. Neoplastic, e.g., Cholangiocarcinoma.
  5. Others, e.g., primary biliary cirrhosis, pregnancy, intrahepatic atresias
- C. Post-hepatic (extrahepatic cholestasis): Various causes are
  1. Intramural: e.g., Stones, parasites.
  2. Mural:
     Congenital, e.g., extrahepatic biliary atresia
     Inflammatory, e.g., acute cholangitis
     Neoplastic, e.g., cholangiocarcinoma, carcinoma of ampulla of Vater.
  3. Extramural:
     Inflammatory, e.g., acute pancreatitis
     Neoplastic, e.g., Carcinoma of pancreas, lymphoma.

Hyperbilirubinemia -- Various causes have been discussed, rise in conjugated or unconjugated bilirubin in blood/serum has been indicated.

Urine urobilinogen - Urobilinogen is normally formed from bilirubin by bacterial action in the bowel. Normally all urobilinogen absorbed from the gut is excreted by the liver, only up to 4 mg appearing in urine in 24 hours.

Urine urobilinogen increased (> 4 mg/24 hours):

(A). Impaired liver function or partial duct obstruction.

(B). 'Overloading' of the liver as a result of increased urobilinogen production following hemolytic disease.

Urine urobilinogen absent:

If biliary duct obstruction is complete, no bilirubin enters the gut, no urobilinogen is formed, and none is found in the urine or feces.

Fecal urobilinogen:

If the hepatobiliary system is functioning, fecal urobilinogen varies directly with rate of red cell hemolysis.

Fecal urobilinogen increased:

Occurs when blood destruction is increased and when biliary obstruction is relieved.In the case of hemolysis, the daily excretion is related to the existing total body hemoglobin mass. If there is a reduced total body hemoglobin mass, accelerated rates of hemolysis may only yield an amount of urobilinogen that would be within normal limits for an individual with normal hemoglobin mass.

Fecal urobilinogen absent:

Occurs with exclusion of bilirubin from the gut in complete biliary tract obstruction and in extreme cases of hepatocellular disease. Absence of urobilinogen in feces is important in indicating biliary tract obstruction; persistent absence is a strong indication of malignant obstructive disease. Decreased fecal urobilinogen excretion may occur when antibiotics which alter intestinal flora are used (tetracyclines, streptomycin, etc).

TROUBLESHOOTING

SPECIMEN PREPARATION

ALL POTENTIALLY INFECTIOUS MATERIAL SHOULD BE HANDLED, LABELED AND TRANSPORTED ACCORDINGLY.

It is essential that the following instructions be followed exactly to assure delivery of a specimen that is adequate for testing. All specimens must be properly identified by indicating the patient's name on every tube or container. The test request form has to be completed and has to include the time and date of the specimen collection, as well as the signature of the Physician requesting the patient's tests.

CHEMISTRY, HEMATOLOGY AND MISCELLANEOUS

Blood: When whole blood is requested, obtain the full amount into a vacuum tube. Lavender, Gray, Green, and Blue Top tubes contain different anticoagulants that inhibit blood coagulation. When drawing these specimens, immediately invert the tube 10-12 times. Do not shake the tube as this can cause Hemolysis. or else dispense required quantity of blood into appropriate non-vacuumed vials and gently mix several times

Serum: Obtain sufficient blood to yield the required volume of Serum. A plain Red Top tube or Red/Mottled top Barrier tube (Corvac, SST, etc.) should be used. When drawing these specimens, immediately invert the tube 5 times. Allow the blood to clot for about 30 minutes and centrifuge for 15 minutes to separate the serum. If a Barrier tube is used, no other manipulations are required. Make sure that the gel has formed a thick, solid, intact barrier between the serum and the clotted cells. If the gel trails into the bottom of the tube, re-centrifuge the tube for another 10 minutes. If a plain Red Top tube or an ordinary bulb is used, transfer the serum with a pipette to a Transfer tube. It is important to avoid hemolysis. Serum in contact with red cells will produce erroneously high Potassium, LDH, and SGOT results and erroneous low Glucose results. Red top tubes for blood banking specimens should not be centrifuged.

Plasma: Treat the specimen as in blood (above)

Urine:

Urinalysis: To adequately test urine specimens the sample should be collected in a tube with a stabilizing chemical present. The tube provided contains a yellow "pop off" cap and a "Stabilur" tablet (or any other stabilizing substance) which preserves the formed elements such as red cells, white cells, casts and epithelial cells. For urinalysis, use a paper cup and transfer about 10 mL of urine to the stopped tube. If no stabilizing substance is used test the urine sample immediately or within an hour at best.

Urine Chemistry: Most assays require a 24 hour collection that should contain boric acid, hydrochloric acid, or sodium carbonate as a preservative. Some analysis require a urine specimen without any additive. Refer to the specific test in this Compendium for specific test details. Instruct the patient to discard the first urine voided upon arising in the morning and thereafter save all urine specimens in the 24 hour container, including the first morning voiding of the following day. Fluid intake during the 24 hour period should be restricted as much as possible. Measure the 24 hour volume and record it on the container and the test request form. Keep the specimen refrigerated until picked up by the laboratory

Urine, Drugs of Abuse (DAU): For routine DAU testing, submit a specimen in a blue "pop-off" capped tube

Urine Culture: Collect the urine into a yellow-label screw capped vial (Boricon). It is not necessary to urinate directly into the vial. It is satisfactory to urinate into a paper cup (non-sterile) and to immediately pour the specimen into the Boricon. Refrigerate the specimen as soon as possible. Otherwise sample may be collected in a sterile urine culture container, if delay is expected, refrigerate (not freeze) the specimen

Frozen Specimens: Certain tests must be submitted frozen because of the stability of the analyte being tested. Keep all frozen specimens separate from the routine tests and submit a separate test request form. As soon as possible separate the serum or plasma and transfer to a plastic transfer tube. Place the specimen in the office freezer and keep until it is solid. Notify the laboratory Logistics Department as soon as possible that you have a frozen specimen for pick up.

PLEASE STORE YOUR SPECIMEN IN THE REFRIGERATOR OR FREEZER UNTIL PICK UP, UNLESS SPECIFICALLY INSTRUCTED TO DO OTHERWISE. Your driver will pick up specimens from the nurse or receptionist at your office or from a box outside your door if after hours.

Cytology: Use Cytology requisition form for all Cytology specimens. Relevant clinical information should be written down in the space provided

Directions for making Direct Smears:

1. Write patient's name with lead pencil on frosted end of clean slide
2. Spread material evenly over slide
3. Fix immediately with cytology spray fixative from a distance of 10 - 12 inches until liquid droplets form
4. Allow slides to dry before sending out in designated slide holders.

Directions for sending Fluids (Collected or Aspirated):

1. Write patient's name on container.
2. All fluids including gastric washings, pleural and peritoneal (ascitic) fluids, have to be placed in a container with an equal volume of 50% ethyl alcohol.
3. Send fluid immediately in securely closed containers.

NOTE: A sputum specimen will be considered unsatisfactory for diagnosis if no pigmented macrophages (dust cells) are present.

Aspiration Biopsy by Fine Needle (FNA):

1. Use the form for Non-Gyn Cytology for all FNA requests. Relevant information and clinical data should be written down as requested, in the space provided.
2. Solid masses: Do direct smears and spray with Cytology spray fixative immediately
3. Fluids: Add directly to fixative supplied in special container.

Method For Obtaining An Optimum Fine Needle Aspiration Specimen:

A high percentage of smears are difficult, and sometimes impossible, to accurately diagnose. This difficulty is primarily due to poorly preserved cellular material or a lack of adequate cellular material. Poorly preserved material is usually due to a delay in fixing the smears or spraying them too closely with the fixative and freezing the material. A lack of adequate cells is generally the result of a hypocellular cystic fluid spread too thinly over the slide

Bio-Reference recommends the following procedure

Fine needle Aspiration Technique

Local anesthesia is not necessary.

1. Clean the skin overlaying the mass with an antiseptic
2. After the needle has entered the mass, retract the plunger to create a vacuum in the syringe
3. Move the needle back and forth several times in the lesion as the material is being sucked into the needle by negative pressure
4. The cell sample should remain in the needle and should not be visible in the syringe barrel
5. Before withdrawing the needle from the lesion, the suction must be released to avoid aspiration of the material into the syringe barrel.

Slide Preparation

6. The needle is quickly detached from the syringe and the plunger is retracted to allow air to fill the syringe
7. Reattach the needle and eject its contents forcefully onto the slides by pushing down on the plunge
8. With the two slide method, place the slides together and gently pull the slides apart with one continuous motion
9. IMMEDIATELY PLACE THE SLIDE(S) IN 50 ML TUBE CONTAINING 95% ALCOHOL to achieve preservation of material
10. After slides are prepared, the syringe is thoroughly rinsed in a separate 50 mL tube of 95% alcohol. Both tubes are then sent along with slides and/or cyst fluid to the laboratory where the specimen is processed using the cytocentrifuge. Using this procedure, even the most hypocellular specimens will generally show a yield of cells which is adequate for diagnosis.

Thin Prep Pap Test Broom-Like Device Protocol

1. Obtain an adequate sampling from the cervix using a broom-like device. Insert the central bristles of the broom into the endocervical canal deep enough to allow the shorter bristles to fully contact the ectocervix. Push gently, and rotate the broom in a clockwise direction five times
2. Rinse the broom into the Preservative Solution vial by pushing the broom into the bottom of the vial 10 times, forcing the bristles apart. As a final step, swirl the broom vigorously to further release material. Discard the collection device
3. Tighten the cap so that the torque line on the cap passes the torque line on the vial.
4. Record the patient's name and ID number on the vial. Record the patient information and medical history on the cytology requisition form.
5. Place the vial and requisition in a specimen bag for transport to the laboratory

...To be continued

BOUQUET

In Lighter Vein

Doctor, doctor, I keep thinking I am a set of curtains!
Pull yourself together, man!

Doctor, doctor, I keep thinking I'm a bell.
Well, just go home and if the feeling persists, give me a ring.

Doctor, doctor, people tell me I'm a wheelbarrow.
Don't let people push you around.

Doctor, doctor, I keep thinking I'm invisible.
Who said that? !

Doctor, doctor, nobody understands me.
What do you mean by that?

Doctor, doctor, People keep ignoring me!
Next!

Doctor, doctor, No one believes a word I say.
Tell me the truth now, what's your REAL problem?

Doctor, doctor, I feel like a pack of cards.
I'll deal with you later.

Doctor, doctor, people keep telling me I'm ugly!
Lay on the couch, face down.

Doctor, Doctor, I can't stop stealing things.
Take these pills for a week; if that doesn't work I'll have a color TV!

Doctor, doctor, I keep thinking I'm a spoon.
Sit there and don't stir.

Doctor, doctor, I'm manic-depressive.
Calm down. Cheer up. Clam down. Cheer up. Calm...

Doctor, doctor, I keep trying to get into fights.
And how long have you had this complaint?
Who wants to know?

Doctor, doctor, I can't concentrate, one minute I'm ok, and the next minute, I'm blank!
And how long have you had this complaint?
What complaint?

Doctor, doctor, I feel so short!
No problem. Hop up on the couch.

Doctor, doctor, I feel like a small bucket.
You do look a little pail.

Doctor, doctor, I've only got 59 seconds to live.
Wait a minute please.

Wisdom Whispers

- "Friendship improves happiness, and abates misery, by doubling our joys, and dividing our grief."
- Our friends interpret the world and ourselves to us, if we take them tenderly and truly."
- "A faithful friend is the medicine of life."
- "We live in deeds, not years: In thoughts not breaths; In feelings, not in figures on a dial. We should count time by heart throbs. He most lives who thinks most, feels the noblest, acts the best."
- "It is easy to hate and it is difficult to love. This is how the whole scheme of things works. All good things are difficult to achieve; and bad things are very easy to get."
- "Without friends, no one would want to live, even if he had all other goods."
- "Having once decided to achieve a certain task, achieve it at all costs of tedium and distaste. The gain in self confidence of having accomplished a tiresome labor is immense."
- "Do not be desirous of having things done quickly. Do not look at small advantages. Desire to have things done quickly prevents their being done thoroughly. Looking at small advantages prevents great affairs from being accomplished."

Brain Teasers

1 Punctate basophilia, Cabot's rings and H.J. bodies are all a feature of
A. Megaloblastic macrocytic anemia. 2. Iron deficiency anemia.
C. Sideroblastic anemia. D. Hereditary spherocytosis

2. Serum LDH would be highest in
A. Megaloblastic macrocytic anemia. B. Iron deficiency anemia.
C. Sideroblastic anemia. D. Hereditary spherocytosis.

3. Hb Bart's is
A. All alpha chains. B. All beta chains C. All gamma chains D. All delta chains

4. Schistocytes would be classically seen in
A. Aplastic anemia B. Microangiopathic anemia C. Iron deficiency anemia D. AIHA

5 Smudge cells are a feature of?
A. CML B. ALL C. CLL D. Hairy cell leukemia

6. Which of the following is not a feature of Multiple Myeloma?
A. Diminished ESR B. Hypercalcemia C. M Band on electrophoresis of serum proteins D. B.J. Proteins in urine

Answers: 1.A, 2.A,3.C,4.B,5.C,6.A