VOLUME - II
ISSUE - XIV
MAR / APR 2006

The Crux

BIMONTHLY FORUM FOR THE LABORATARIANS

CONTENTS

Editorial

The current trend of getting accreditations has caught on like wild fire. Every hospital or discerning laboratory is running and gunning for obtaining some or the other way of assuring its clients that they use world-class standards in their respective departments. Medical Laboratories being the backbones of all healthcare delivery systems cannot be left behind. One wrong report - and you have harmed the patient, perhaps irreversibly. What goes along with it is your reputation that took years, may be decades to establish. Why let it go when a little care and minimum expense can help preserve the same.

Our previous issues have at length dealt with Quality Assurance norms in hematology and biochemistry laboratories; this issue brings to you Quality Assurance aspects as related to a microbiology laboratory.

Read on the TROUBLE SHOOTING portion to get to the bottom of it in order to reach to the top in the diagnostic fraternity. Seeing is believing, if you can actually isolate and identify the culprit organism, you firmly establish the diagnosis and thereafter provide a battery of curative antibiotics. Results are visible within days in most cases. In our forthcoming issues we shall endeavour to cover Quality Assurance aspects of all sub-specialities of a pathology laboratory.

The DISEASE DIAGNOSIS segment divulges all about pancreatitis. Acute and chronic pancreatitis being distinct entities are considered separately. Etiopathogenesis, diagnostic and prognostic parameters alongwith differential diagnosis and a bit of therapeutics is presented. To differentiate from amylase rise on account of salivary gland disorder you can now actually estimate the different isoenzymes of amylase. What are the other pancreatic function tests available is also given in the article.

Are their only lipids to be considered while estimating coronary artery disease risk? Are their any non-lipid parameters too? Is their anything beyond the traditional lipid panel? The INTERPRETATION folio shall crystallize your thoughts and views about predicting coronary vascular disease or CVD. How can CRP, TLC, homocysteine or BNP assist you in predicting future CVD?

All your queries shall be answered and interpreted more than adequately in the INTERPRETATION section. What should be the goals of instituted therapy are also mentioned clearly.

All work and no play! How's that possible? Flip open BOUQUET please.

ISO 9001

Tulip Group

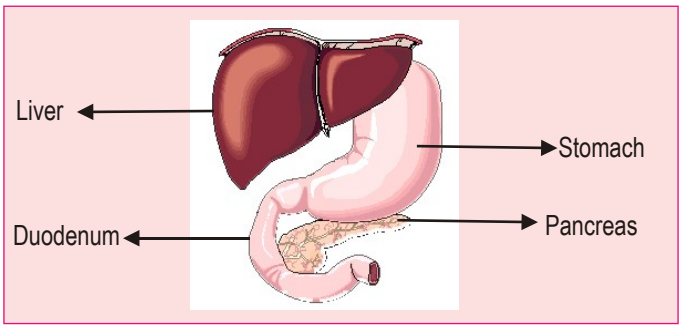
DISEASE DIAGNOSIS

PANCREATITIS

Introduction

Pancreatitis is an inflammatory process in which pancreatic enzymes auto digest the gland.

The gland can sometimes heal without any impairment of function or any morphologic changes. This process is known as acute pancreatitis. It can recur intermittently, contributing to the functional and morphologic loss of the gland. Recurrent attacks are referred to as chronic pancreatitis. Both forms of pancreatitis are present in the ED with acute clinical findings.



Thus pancreatitis can be divided into acute and chronic forms. Both are discussed separately below.

Pathophysiology in general

Because the pancreas is located in the retroperitoneal space with no capsule, inflammation can spread easily. In acute pancreatitis, parenchymal edema and peripancreatic fat necrosis occur first. This process is known as acute edematous pancreatitis.

When necrosis involves the parenchyma, accompanied by hemorrhage and dysfunction of the gland, the inflammation evolves into hemorrhagic or necrotizing pancreatitis.

Pseudocysts and pancreatic abscesses can result from necrotizing pancreatitis because of enzymes being walled off by granulation tissue (ie, pseudocyst formation) or bacterial seeding of pancreatic or peripancreatic tissue (ie, pancreatic abscess formation). An ultrasound or, preferably, a CT scan can be used to detect both.

The inflammatory process can cause systemic effects because of the presence of cytokines, such as bradykinins and phospholipase A. These cytokines may cause vasodilation, increase in vascular permeability, pain, and leukocyte accumulation in the vessel walls. Fat necrosis may cause hypocalcemia. Pancreatic B cell injury may lead to hyperglycemia.

Mortality/Morbidity

Although acute pancreatitis should be noted, chronic pancreatitis has a more severe presentation as episodes recur.

Acute respiratory distress syndrome (ARDS), acute renal failure, cardiac depression, hemorrhage, and hypotensive shock all may be systemic manifestations of acute pancreatitis in its most severe form.

Sex

No predilection exists.

Age

The risk for persons aged 35-64 years is 10 times higher than for any other group.

Acute Pancreatitis

Etiology and Pathogenesis

Biliary tract disease and alcoholism account for 80% of hospital admissions for acute pancreatitis. The remaining 20% are attributed to drugs (eg, azathioprine, sulfasalazine, furosemide, valproic acid), estrogen use associated with hyperlipidemia, infection (e.g, mumps), hypertriglyceridemia, endoscopic retrograde pancreatography, structural abnormalities of the pancreatic duct (eg, stricture, cancer, pancreas divisum), structural abnormalities of the common bile duct and ampullary region (e.g, choledochal cyst, sphincter of Oddi stenosis), surgery (particularly of stomach and biliary tract and after coronary artery bypass grafting), vascular disease (especially severe hypotension), blunt and penetrating trauma, hyperparathyroidism and hypercalcemia, renal transplantation, hereditary pancreatitis, or uncertain causes.

In biliary tract disease, attacks of pancreatitis are caused by temporary impaction of a gallstone in the sphincter of Oddi before it passes into the duodenum. The precise pathogenetic mechanism is unclear; recent data indicate that obstruction of the pancreatic duct in the absence of biliary reflux can produce pancreatitis, suggesting that increased ductal pressure triggers pancreatitis.

Alcohol intake > 100 g/day for several years may cause the protein of pancreatic enzymes to precipitate within small pancreatic ductules. In time, protein plugs accumulate, inducing additional histologic abnormalities. After 3 to 5 yr, the first clinical episode of pancreatitis occurs, presumably because of premature activation of pancreatic enzymes.

Edema or necrosis and hemorrhage are prominent gross pathologic changes. Tissue necrosis is caused by activation of several pancreatic enzymes, including trypsin and phospholipase A_2. Hemorrhage is caused by extensive activation of pancreatic enzymes, including pancreatic elastase, which dissolves elastic fibers of blood vessels. In edematous pancreatitis, inflammation is usually confined to the pancreas, and the mortality rate is < 5%. In pancreatitis with severe necrosis and hemorrhage, inflammation is not confined to the pancreas, and the mortality rate is 10 to 50%.

Pancreatic exudate containing toxins and activated pancreatic enzymes permeates the retroperitoneum and at times the peritoneal cavity, inducing a chemical burn and increasing the permeability of blood vessels. This causes extravasation of large amounts of protein-rich fluid from the systemic circulation into "third spaces," producing hypovolemia and shock. On entering the systemic circulation, these activated enzymes and toxins increase capillary permeability throughout the body and may reduce peripheral vascular tone, thereby intensifying hypotension. Circulating activated enzymes may damage tissue directly (e. g, phospholipase A_2 is thought to injure alveolar membranes of the lungs).

Symptoms and Signs

In pancreatitis, pancreatic enzymes activate complement and the inflammatory cascade, thus producing cytokines. Patients typically present with fever and an elevated WBC count. It may thus be difficult to determine if infection is the cause or has developed during the course of pancreatitis.

Most patients suffer severe abdominal pain, which radiates straight through to the back in about 50%; rarely, pain is first felt in the lower abdomen. Pain usually develops suddenly in gallstone pancreatitis versus over a few weeks in alcoholic pancreatitis. Pain is severe, often requiring large doses of parenteral narcotics. The pain is steady and boring and persists without relief for many hours and usually for several days. Sitting up and leaning forward may reduce pain, but coughing, vigorous movement, and deep breathing may accentuate it. Most patients experience nausea and vomiting, at times to the point of dry heaves.

The patient appears acutely ill and is sweating. Pulse rate is usually 100 to 140 beats/min. Respirations are shallow and rapid. BP may be transiently high or low, with significant postural hypotension. Temperature may be normal or even subnormal at first but may increase to 37.7 to 38.3° C (100 to 101° F) within a few hours. Sensorium may be blunted to the point of semicoma. Scleral icterus is occasionally present. Examination of the lungs may reveal limited diaphragmatic excursion and evidence of atelectasis.

About 20% of patients experience upper abdominal distention caused by gastric distention or a large pancreatic inflammatory mass displacing the stomach anteriorly. Pancreatic duct disruption may cause ascites (pancreatic ascites). Abdominal tenderness always occurs and is often severe in the upper abdomen and less severe in the lower abdomen. Mild-to-moderate muscular rigidity may exist in the upper abdomen but is rare in the lower abdomen. The entire abdomen rarely exhibits severe peritoneal irritation in the form of a rigid boardlike abdomen. Bowel sounds may be hypoactive. Rectal examination usually discloses no tenderness,

and the stool usually tests negative for occult blood.

Complications

Death during the first several days of acute pancreatitis is usually caused by cardiovascular instability (with refractory shock and renal failure) or respiratory failure (with hypoxemia and at times adult respiratory distress syndrome) and occasionally by heart failure (secondary to unidentified myocardial depressant factor). Circulating enzymes and toxins are thought to play a large role in early death.

Death after the first week is usually caused by pancreatic infection or pancreatic pseudocyst.

Pancreatic infection of devitalized retroperitoneal tissue is usually caused by gram-negative organisms. Infection should be suspected if the patient maintains a generally toxic appearance with elevated temperature and WBC count or if deterioration follows an initial period of stabilization. The diagnosis is supported by positive blood cultures and particularly by the presence of air bubbles in the retroperitoneum on abdominal CT. Percutaneous aspiration of pancreatic exudate guided by abdominal CT may reveal organisms on Gram stain or culture, which should lead to prompt surgical debridement. Mortality rate is usually 100% without extensive surgical debridement of infected retroperitoneal tissue.

A **pancreatic pseudocyst** is a collection of enzyme-rich pancreatic fluid and tissue debris arising within areas of necrosis or an obstructed smaller duct. It is not surrounded by a true capsule. Death is caused by secondary infection, hemorrhage, or rupture.

Diagnosis

Acute pancreatitis should be considered in the differential diagnosis of every acute abdomen. The differential diagnosis of acute pancreatitis includes a perforated gastric or duodenal ulcer, mesenteric infarction, strangulating intestinal obstruction, ectopic pregnancy, dissecting aneurysm, biliary colic, appendicitis, diverticulitis, inferior wall MI, and hematoma of abdominal muscles or spleen.

Laboratory tests cannot confirm a diagnosis of acute pancreatitis but can support the clinical impression. **Serum amylase and lipase concentrations** increase on the first day of acute pancreatitis and return to normal in 3 to 7 days. Both may remain normal if destruction of acinar tissue during previous episodes precludes release of sufficient amounts of enzymes to raise serum levels. Serum amylase may remain normal if there is coexisting hypertriglyceridemia (which may contain a circulating inhibitor that must be diluted before an elevation in serum amylase can be detected). Both serum amylase and lipase may be increased in other disorders, such as renal failure and abdominal conditions requiring urgent surgical therapy (eg, perforated ulcer, mesenteric vascular occlusion, intestinal obstruction associated with ischemia). Other causes of increased serum amylase include salivary gland dysfunction, macroamylasemia, and tumors that secrete amylase.

The **amylase: creatinine clearance ratio** does not appear to have sufficient sensitivity or specificity to confirm a diagnosis of pancreatitis. It is generally used to diagnose macroamylasemia when no pancreatitis truly exists. In macroamylasemia, amylase bound to serum immunoglobulin falsely elevates the serum amylase level. Fractionation of total serum amylase into pancreatic type (p-type) and salivary-type (s-type) isoamylase is now possible in most commercial laboratories. p-Type increases on the first day of pancreatitis and, along with serum lipase, remains elevated longer than total serum amylase. However, p-type also increases in renal failure and in other severe abdominal conditions in which amylase clearance is altered.

The WBC count usually increases to 12,000 to 20,000/µL. Third space fluid losses may increase the Hct to as high as 50 to 55%, indicating severe inflammation. Hyperglycemia may occur. Serum Ca concentration falls as early as the first day because of the formation of Ca "soaps" secondary to excess generation of free fatty acids, especially by pancreatic lipase. Serum bilirubin increases in 15 to 25% of patients because pancreatic edema compresses the common bile duct.

Supine and upright plain x-rays of the abdomen may disclose calculi within pancreatic ducts (evidence of prior inflammation and hence chronic pancreatitis), calcified gallstones, or localized ileus in the left upper quadrant or central abdomen (a "sentinel loop" of small bowel, dilation of the transverse colon, or duodenal ileus). **Chest x-ray** may reveal atelectasis or a pleural effusion (usually left-sided or bilateral but rarely confined to the right pleural space).

Ultrasound should be performed; it may detect gallstones or dilation of the common bile duct, indicating biliary tract obstruction. Edema of the pancreas may be visualized, but overlying gas frequently obscures the pancreas. **CT** usually offers better visualization of the pancreas (unless the patient is very thin). **CT** is recommended for severe pancreatitis or if a complication ensues (e.g, hypotension or progressive leukocytosis and elevation of temperature). Although > 80% of patients with gallstone pancreatitis pass the stone spontaneously, ERCP with sphincterotomy and stone removal is indicated for patients who do not improve over the initial 24 hours of hospitalization. Patients who spontaneously improve generally undergo elective laparoscopic cholecystectomy. Elective cholangiography in these patients remains controversial. However, the advent of MRI cholangiography may make imaging of the biliary tree noninvasive and simple.

Differential Diagnosis

Aneurysm, abdominal Cholangitis, Cholecystitis and Biliary Colic, Cholelithiasis, Gastroenteritis, Hepatitis, Mesenteric Ischemia, Obstruction-Large Bowel, Obstruction-Small Bowel

Other Problems to be Considered

Perforated viscus, Acute peritonitis, Choledocholithiasis, Macroamylasemia, Macrolipasemia, Intestinal obstruction, Pancreatic cancer, Malabsorption syndromes/processes

Prognosis

Ranson's 11 prognostic signs help estimate the prognosis of acute pancreatitis. Five signs can be documented at admission: age > 55 yr, serum glucose > 200 mg/dL (> 11.1 mmol/L), serum LDH > 350 IU/L, AST > 250 U, and WBC count > 16,000/µL. The rest are determined within 48 h after admission: Hct decrease > 10%, BUN rise > 5 mg/dL (> 1.8 mmol Urea/L), serum Ca < 8 mg/dL (< 2 mmol/L), PaO_2 < 60 mm Hg, base deficit > 4 mEq/L, and estimated fluid sequestration > 6 L. Mortality increases with the number of positive signs: If fewer than three signs are positive, the mortality rate is < 5%; if three or four are positive, it is 15 to 20%.

Pancreatitis associated with necrosis and hemorrhage has a mortality rate 10 to 50%. This diagnosis is suggested by a progressive decrease in Hct, presence of hemorrhagic fluid within ascites, reduction in serum Ca, and **Grey Turner's** or **Cullen's sign** (indicating extravasation of hemorrhagic exudate to the flanks or umbilical region, respectively).

If CT shows only mild pancreatic edema, the prognosis is excellent; a markedly swollen pancreas denotes a more severe prognosis, especially when extravasation of fluid (eg, within retroperitoneal spaces and lesser sac) or pancreatic necrosis is evident. Addition of IV contrast aids recognition of pancreatic necrosis because loss of integrity of the microcirculation reduces parenchymal perfusion; thus, the lesion does not enhance with contrast. However, if the swollen pancreas is edematous but its microcirculation is intact, there is uniform enhancement of the parenchyma.

Pancreatic necrosis is associated with increased morbidity, mortality, and likelihood of infection. IV contrast agents should be used cautiously if there is renal impairment. Also, experimental data indicate that the use of IV contrast agents during the onset of acute pancreatitis may cause necrosis in areas of low perfusion (i.e, ischemia). Thus, contrast-enhanced CT should be performed only after a patient has been adequately hydrated.

Treatment

Emergency Department Care

Most of the cases presenting to the ED are treated conservatively, and approximately 80% respond to such treatment.

Fluid resuscitation

- Monitor accurate intake/output and electrolyte balance of the patient.
- Crystalloids are used, but other infusions, such as packed red blood cells (PRBCs), are occasionally administered, particularly in the case of hemorrhagic pancreatitis. Central lines and Swan-Ganz catheters are used in patients with severe fluid loss and very low blood pressure.

Patients should have nothing by mouth, and a nasogastric tube should be

inserted to assure an empty stomach and to keep the GI system at rest.
Begin parenteral nutrition if the prognosis is poor and if the patient is going to be kept in the hospital for more than 4 days.
Analgesics are used to relieve pain. Meperidine is preferred over morphine because of the greater spastic effect of the latter on the sphincter of Oddi.
Antibiotics are used in severe cases associated with septic shock or when the CT scan indicates that a phlegmon of the pancreas has evolved.
Other conditions, such as biliary pancreatitis associated with cholangitis, also need antibiotic coverage. The preferred antibiotics are the ones secreted by the biliary system, such as ampicillin and third generation cephalosporins.
Continuous oxygen saturation should be monitored by pulse oximetry and acidosis should be corrected. When tachypnea and pending respiratory failure develops, intubation should be performed.
Perform CT-guided aspiration of necrotic areas, if necessary.
An ERCP may be indicated for common duct stone removal.

Consultations

Consult a general surgeon in the following cases:
For phlegmon of the pancreas, surgery can achieve drainage of any abscess or scooping of necrotic pancreatic tissue. It should be followed by postoperative lavage of the pancreatic bed.
In patients with hemorrhagic pancreatitis, surgery is indicated to achieve hemostasis, particularly because major vessels may be eroded in acute pancreatitis.
Patients who fail to improve despite optimal medical treatment or patients who push the Ranson score even further are taken to the operating room. Surgery in these cases may lead to a better outcome or confirm a different diagnosis. In biliary pancreatitis, a sphincterotomy (i.e, surgical emptying of the common bile duct) can relieve the obstruction. A cholecystectomy may be performed to clear the system from any source of biliary stones.

Chronic Pancreatitis

Etiology and Pathogenesis

Chronic pancreatitis most commonly results from alcoholism and idiopathic causes. Similar to acute pancreatitis, microlithiasis has been implicated in some cases of chronic pancreatitis. Rare causes are hereditary pancreatitis, hyperparathyroidism, and obstruction of the main pancreatic duct caused by stenosis, stones, or cancer. Rarely, severe acute pancreatitis causes sufficient pancreatic ductal stenosis to impair drainage and result in chronic pancreatitis. In India, Indonesia, and Nigeria, idiopathic calcific pancreatitis occurs among children and young adults.

Symptoms and Signs

Symptoms and signs may be identical to those of acute pancreatitis. Although there is occasionally no pain, severe epigastric pain may last many hours or several days. Possible causes include acute inflammation not recognized by conventional tests, distention of pancreatic ducts caused by strictures or calculi, a pseudocyst, perineural inflammation, or obstruction of either the duodenum or the common bile duct caused by fibrosis of the head of the pancreas. Abdominal pain may subside as acinar cells that secrete pancreatic digestive enzymes are progressively destroyed. When lipase and protease secretions are reduced to <10% of normal, the patient develops steatorrhea, passing greasy stools or even oil droplets, and creatorrhea. Islet cell destruction reduces insulin secretion and causes glucose intolerance.

Diagnosis

Laboratory tests, including amylase and lipase, are frequently normal, probably because of significant loss of pancreatic function. Markers of inflammation (e.g, WBC count) are generally minimally elevated as well.

Structural abnormalities can be visualized by plain x-ray of the abdomen (showing pancreatic calcification, which indicates intraductal stones), abdominal ultrasound or CT (showing abnormalities in size and consistency of the pancreas, pancreatic pseudocyst, or dilated pancreatic ducts), and ERCP (showing abnormalities of the main pancreatic duct and secondary branches). However, these imaging studies may be normal in the first few years of disease.

Tests of pancreatic function assess endocrine and exocrine function. Diabetes mellitus is present if a 2-hr postprandial serum glucose level is > 200 mg/dL (> 11.1 mmol/L) or two fasting plasma glucose levels are > 126mg/dL (> 7 mmol/L).
The most sensitive test of pancreatic exocrine function, the secretin test, is unavailable in most hospitals. It involves positioning a tube in the duodenum and collecting pancreatic secretions stimulated by IV secretin alone or with either cholecystokinin or cerulein. Duodenal contents are collected for volume determination, HCO_3 concentration, and enzyme concentration. A collection that is of normal volume (> 2 mL/kg) and low in HCO_3 (< 80 mEq/L) suggests chronic pancreatitis; low volume (< 2 mL/kg), normal HCO_3 (> 80 mEq/L), and normal enzyme levels suggest pancreatic duct obstruction, perhaps secondary to tumor, and should prompt ERCP.

A 72-hr test for stool fat is insensitive for pancreatic exocrine dysfunction because steatorrhea does not occur until lipase output is < 10% of normal. Other, more sensitive tests include measurement of serum trypsinogen, fecal chymotrypsin, and urinary *p*-aminobenzoic acid (bentiromide test).

Treatment

A relapse of chronic pancreatitis may require treatment similar to that of acute pancreatitis. The patient must eschew alcohol. At times, IV fluids and fasting prove beneficial. Dietary measures of uncertain benefit include small feedings restricted in fat and protein (to reduce secretion of pancreatic enzymes) and an H_2 blocker or antacids (to reduce acid-stimulated release of secretin, increasing the flow of pancreatic juice). Too often, these measures do not relieve pain, requiring increased amounts of narcotics, with the threat of addiction. Medical treatment of chronic pancreatic pain is often unsatisfactory.
There has been recent interest in the use of potent pancreatic enzymes to treat chronic pain because enzymes given in quantity inhibit the release of cholecystokinin from the duodenal mucosa, thereby reducing the secretion of pancreatic enzymes. The recommended dose of oral pancreatic enzymes is 30,000 U of lipase (e.g, six tablets of pancrelipase) with each meal. The use of pancreatic extracts to ameliorate chronic pancreatic pain appears to be more successful in mild idiopathic pancreatitis than in alcoholic pancreatitis. Because the duodenum requires high-dose enzymes, sustained-release preparations are not effective in relieving pain. Octreotide, a long-acting somatostatin analog, has also been examined to "rest" the pancreas. However, pain relief appears minimal.
A pancreatic pseudocyst, which may cause chronic pain, can be decompressed into a nearby structure to which it firmly adheres (e.g, the stomach) or into a defunctionalized loop of jejunum (via a Roux-en-Y cystojejunostomy) to which it does not. If the pain is refractory and the main pancreatic duct is dilated (diameter > 8 mm), a lateral pancreaticojejunostomy (Puestow procedure) relieves pain in about 70 to 80% of patients. If the duct is not dilated, a resection can be considered, eg, distal pancreatectomy (for extensive disease at the tail of the pancreas) or Whipple's operation (for extensive disease at the head of the pancreas). These operative approaches may relieve pain in 60 to 80% of patients and should be reserved for patients with a nondilated duct who have discontinued using alcohol and who can manage diabetes that may be intensified by pancreatic resection.In general, more extensive pancreatic resections (e.g, 95% subtotal distal pancreatectomy) have been abandoned. As an alternative to surgery, percutaneous denervation of the celiac plexus with alcohol or with a combination of lidocaine and corticosteroids can provide transient pain relief. Steatorrhea can be improved, but rarely abolished with four to six tablets of potent pancreatic extracts (each tablet or capsule contains lipase 5000U) with meals.

Gallstones and Pancreatitis

Gallstones can cause pancreatitis and they usually require surgical removal. Ultrasound or a CAT scan can detect gallstones and can sometimes give an idea of the severity of the pancreatitis. When gallstone surgery can be scheduled depends on how severe the pancreatitis is. If the pancreatitis is mild, gallstone surgery may proceed within about a week. More severe cases may mean gallstone surgery is delayed for a month or more. After the gallstones are removed and inflammation goes away, the pancreas usually returns to normal.

Pancreatitis in Children

Chronic pancreatitis is rare in children. Trauma to the pancreas and hereditary pancreatitis are two known causes of childhood pancreatitis. Children with cystic fibrosis, a progressive, disabling, and incurable lung disease, may also have pancreatitis. But more often the cause is not known.

INTERPRETATION

INTERPRETATION OF LIPID & NON LIPID MARKERS IN CARDIO-VASCULAR DISEASE (CVD)

INTRODUCTION

Risk factors such as age, sex, family history, smoking, obesity, and the presence or absence of elevated blood pressure, diabetes, or dyslipidemia have been used to assess a patient's risk for cardiovascular disease. Despite the link between these variables and heart disease, cholesterol blood tests are often within normal limits. Also, some patients who have no identifiable risk factors often develop significant undetected cardiovascular disease.

As a result, alternative markers can aid in the detection of cardiovascular disease and the risk for cardiovascular disease. There are three markers ultra sensitive CRP-US also known as high-sensitivity C-reactive protein (hs-CRP), homocysteine, and brain natriuretic peptide (BNP) as well as other nontraditional lipid values that can be used in the assessment, evaluation, and management of dyslipidemias and heart disease.

Testing for CRP-US

The presence of C-reactive protein can indicate general, systemic inflammation; CRP-US is specific for endothelial inflammation. Studies have shown that an abnormally elevated CRP-US is an independent predictor of future cardiovascular events in adults.

Although the reference ranges provided by the laboratory performing the test should be followed, CRP-US levels of < 1.0 mg/L are considered low risk, 1.0 to 3.0 mg/L are considered average-to-moderate risk, and 3.1 to 10.0 mg/L are considered high risk for future cardiac events. If the CRP-US remains elevated (>10.0 mg/L) after treatment, it may represent noncardiovascular inflammation and further investigation is warranted. If the CRP-US is elevated and other risk factors are present, the provider may consider pharmacologic therapy.

Aspirin and statin therapy may reduce the cardiac-specific inflammation, thus reducing the risk of future cardiovascular events. The elevated CRP-US is initially treated with aspirin therapy, usually initiated with enteric-coated aspirin at 81 mg/day. The CRP-US level generally responds to 81 mg enteric-coated ASA/day; if not, the dose may be increased to 1 enteric-coated 325 mg ASA/day.
Evaluating dyslipidemia therapy may be necessary if the CRP-US does not return to normal levels. Although there are no international guidelines regarding the frequency of CRP-US testing, it may be tested with the initial lipid panel (total cholesterol, high-density lipoprotein [HDL], low-density lipoprotein [LDL], triglycerides, total cholesterol/ HDL ratio). If the CRP-US is elevated and diet and pharmacologic therapy are ongoing, the CRP-US level can be checked every 3 months until satisfactory levels are achieved. Once normal values of CRP-US are sustained, it can be checked at 6 months, 12 months, and then annually.

Leukocyte Count

Testing for leukocyte count is another means of measuring systemic inflammation. A recent study indicates that a higher leukocyte count in women between 50 and 79 years of age is suggestive of future cardiovascular events. Women with leukocyte counts > 6.71 x 10^9 cells/L had more than twice the risk of cardiovascular events, such as fatal coronary heart disease, non-fatal myocardial infarction, and stroke than those whose counts ranged from 2.5 to 4.7 x 10^9 cells/L. Leukocyte count is an inexpensive, widely available measure of systemic inflammation that along with CRP-US may assist further in identifying high-risk individuals.

Testing Homocysteine Levels

Elevated homocysteine levels have been identified as a strong, independent risk factor for cardiovascular disease. Regulation of homocysteine, a sulfur-containing amino acid, can depend on the patient's diet. A deficiency in folate, vitamins B_6 and B_{12}, can cause homocysteine levels to rise. When homocysteine levels are elevated, blood clotting may increase and the vascular endothelium and smooth muscle cells may be damaged. Thrombogenesis also causes platelet aggregation and turbulent blood flow. Recent reports confirm that an elevated serum homocysteine level is associated with an increase in the risk of the development of coronary artery disease in adults. Just as for CRP-US, there are no international guidelines for the use of plasma homocysteine levels in a treatment plan. A homocysteine level can be obtained, along with the initial lipid panel and CRP-US, in any patient with a current diagnosis of dyslipidemia or a strong family history of coronary heart disease.

Based on the laboratory reference range for normal limits (< 11.4 umol/L for males and < 10.4 umol/L for females), an elevated homocysteine level can be treated with diet and pharmacologic therapy.]

A diet rich in folic acid (1 to 2 mg/day) and vitamin B complex (especially B_6 and B_{12}) may lower elevated homocysteine levels. Citrus fruits, tomatoes, vegetables, and grain products are all good sources of folic acid and vitamin B. Patients should be instructed to read nutrition labels to ensure adequate intake of folic acid and vitamin B.

Prenatal vitamins contain about 1 mg of folic acid and preparations are available for the treatment of hyper-homocysteinemia that contain between 1 and 2.5 mg of folic acid along with vitamins B_6 and B_{12}.

Homocysteine levels should be checked every 3 months until normal levels are achieved; then every 6 to 12 months. Once therapy is initiated and maintained, normal values of homocysteine are often reached within 3 to 6 months.

TABLE 1: Cardiac Risk Markers in Adults

Test	Reference Values	Interpretation/Comments
Ultra-sensitive C-reactive protein (CRP-US)	1.0 mg/L 1.0 - 3.0 mg/L 3.1 -10.0 mg/L	Low risk Average/ moderate risk High risk
Homocysteine	4.3 - 11.4 mol/L 3.3 - 10.4 mol/L	Males older than 17 yrs Females older than 17 yrs
Brain natriuretic peptide (BNP)	100 pg/mL 100 pg/mL	Within normal limits Abnormal
Leukocyte	6.71 x 10^9 cells/L	Postmenopausal women
Non HDL-C[2]	130 mg/dL 160 mg/dL	Diabetics Non diabetics, or patients at risk
Very low density Lipoprotein[3](VLDL)	10 mg/dL	
Intermediate-density Lipoprotein[4](IDL/ILDL)	20 mg/dL	
Apoprotein A (Apo-A/LpA)	LpA-I 0.40-1.00 g/L 0.41-1.22 g/L	 Males Females
High-density lipoprotein-2b[5](HDL_{2b}) Lp(a)[6]	10 mg/dL 10 mg/dL	
Apolipoprotein B[7]	90 mg/dL 80 mg/dL[8]	Does not require fasting

1. Discuss availability of these tests with the laboratory; reference ranges may vary
2. Correlates highly with LpB levels
3. Main lipoprotein-carrying triglycerides
4. Between VLDL & IDL ; more atherogenic than LDL; under strong genetic control
5. Antiatherogenic; risk decreased
6. Atherogenic
7. Found in VLDL, IDL, LDL
8. For patients at high-risk or patients with coronary artery disease

Testing Brain Natriuretic Peptide

Brain natriuretic peptide is one of a group of amino acid polypeptides that help to regulate renal and cardiovascular function. Brain natriuretic peptide, a hormone first identified in the brain, is present in the heart, primarily the ventricular myocardium. When atrial and ventricular dilation are present, as in congestive heart failure (CHF), BNP helps to decrease the workload of the heart by counteracting vaso-constriction and sodium retention. In patients with CHF that has developed as a result of long-standing and untreated cardiovascular disease, elevated levels of BNP may be used to reflect the severity of CHF. This marker may aid in the diagnosis, evaluation, and management of patients with both cardiovascular disease and CHF. Laboratory reference ranges may vary, but levels > 100 pg/mL are considered abnormal and a diagnosis of CHF or left ventricular dysfunction is likely. There are no international guidelines that specify when to monitor BNP values or how often testing is needed. It is reasonable to order a BNP for a patient with a history of long-standing or poorly treated hypertension or dyslipidemia in which CHF is suspected. In addition, this test may be given to patients experiencing shortness of breath to differentiate cardiovascular etiology from respiratory etiology. Brain natriuretic peptide may be ordered for a patient who presents in either primary or tertiary care settings. Follow-up evaluations of BNP are appropriate to evaluate patient progress and the effectiveness of the treatment plan.

(...To be continued)

BOUQUET

In Lighter Vein

A guy burned two ears so they were asking him at the hospital how it happened.
He said, "I was ironing my clothing and the phone rang...so instead of the phone I picked up the iron and burned my ear..."
“But how the heck did you burn the other ear?" The doctor asked.
"How do you think I called you people?”

One day, Mr. Phillard rushed his pregnant wife over to the hospital. As the doctors were prepping his wife, Mr. Phillard's idiot brother Bill arrived to watch the birth. But when Mr. Phillard saw the blood and everything else, he fainted. When Mr. Phillard woke up he was in a bed with the doctor standing above him. "Mr. Phillard," the doctor said, "you are in the recovery room. Don't worry, your wife is fine and she had twins, a boy and a girl. Because you were unconscious and your wife was still under anaesthesia, she requested that your brother Bill name the kids."
"What! My brother, the idiot! I can't believe you let him! What did he name them?"
"He named your daughter Denise."
"Hey, not bad! I underestimated my brother. What did he name my son?"
"He named your son Denephew.”

Three elderly ladies were at the doctor for a cognitive reasoning test. The doctor says to the first gal, "What is three times three?" "297," was her prompt reply. "Ummm humm," says the doc.
The doctor says to the second lady, "It's your turn now. What is three times three?" "Friday," replies the second lady. "Ummm humm..."
Then the doc says to the third, "Okay, mam, your turn. What's three times three?"
"Nine," she says. "That's wonderful!" says the doc. "Tell me, how did you get that?"
"Simple," she says, beaming... "I subtracted 297 from Friday!"

A man approached his family physician and said, "Doc, I'm afraid you'll have to remove my wife's tonsils one of these days."
The doctor pulled out the family's medical file and exclaimed, "Why, I removed them six years ago! Did you ever hear of a woman having two sets of tonsils?"
"No," the husband retorted, "but you've heard of a man having two wives, haven't you?"

Doctor! I have a serious problem . I can never remember what I just said.
When did you first notice this problem?
Scott: What problem.

What do you call a sheep with no legs?
A cloud.

Wisdom Whispers

He who has a thousand friends has not a friend to spare. And he who has one enemy will meet him everywhere

Friendship make prosperity more shining and lessens adversity by dividing and sharing it.

Without friends no one would choose to live, though he had all other goods.

The shifts of Fortune test the reliability of friends.

Do not protect yourself by a fence, but rather by your friends.

You can make more friends in two months by becoming interested in other people than you can in two years by trying to get other people interested in you.

Never refuse any advance of friendship, for if nine out of ten bring you nothing, one alone may repay you.

Old mothers used to say that there are no strangers, only friends you haven't met yet.

Never explain--your friends do not need it and your enemies will not believe you anyway.

In prosperity our friends know us; in adversity we know our friends.

Brain Teasers

1. The enzyme GGT is related to which of the following?
   A. Hepatobiliary disease B. Organophosphorus poisoning C. AMI
   D. Pancreas

2. Which of the following enzymes can be related to MI, parenchymal liver disease and skeletal muscle disease?
   A. ALP B. ALT C. AST D. CK

3. Which of the following enzymes can be related to Organo phosphorus poisoning?
   A. Cholinesterase B. LD C. Amylase D. Lipase

4. DGKC is a method utilised to estimate which of th following constituents in the blood?
   A. ALP B. Bilirubin C. Calcium D Inorganic phosphorus

5. Angiotensin converting enzyme is usually estimated to diagnose which of the following disorders?
   A. MI B. Sarcoidosis C. Leptospirosis D. Dengue fever

6. How many isoenzymes has CK got?
   A. 2 B. 3 C. 4 D. 8

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

TROUBLE SHOOTING

QUALITY ASSURANCE IN BACTERIOLOGY

QUALITY CONTROL OF MEDIA AND STAINS

Culture Media are used in the laboratory for a variety of purposes. These are used to support the growth of microorganisms showing typical colonial and morphological appearance.
Media are also used to demonstrate many other properties of organisms, e.g. production of acid and gas in carbohydrate fermentation media or haemolysis on blood agar. Variations in the composition of the medium may alter these characters.

Quality Control of Media

Sources of Media

A few years back media used to be prepared from basic chemical ingredients, but laboratories are no longer required to do this now.

Dehydrated Media

These are commercially available and require only the addition of water to be reconstituted for use. The responsibility for quality control lies with the manufacturer.

However, it has to be tested for its quality, after preparation, because of changes that can be brought about by the process of reconstitution and sterilization.

Dehydration with Additive

For isolation of fastidious organisms, certain additives need to be used when media are prepared in the laboratory. The additives usually are unstable materials such as blood, serum or other growth factors. Hence, quality control needs to be maintained.

Commercially Prepared Media

Ready to use media are commercially available. In these media also the responsibility for quality control maintenance lies with the manufacturer but laboratories need to keep a watch on their behaviour.

Sources of Error

Inappropriate Medium

Since dehydrated media are usually arranged alphabetically on a shelf, one may select the wrong bottle inadvertently, or an improper additive might be selected, making the medium unsuitable for use.

It is always important to read the label, particularly when a new lot of medium has been received in the laboratory.

Water

Measure carefully the amount of water that is added when reconstituting media. Since impurities render tap water unsuitable for the preparation of most biological media, laboratories should use either distilled water, deionized water, or water that has been treated in both ways.

Weighing

Accurate balances should be used for weighing dry materials. Weighing errors significantly alter the composition of the final product.

Dispensing

Media should be dispensed accurately and aseptically in plates and tubes. Failure to measure the amount accurately may result, for example, in too shallow or too deep agar medium, either of which may make the medium unsuitable for use.

Proper Sterilization

A common error in media preparation is sterilizing media at too high a temperature or for too long a period, or both. This may result in deterioration or decomposition of some constituents of the media, which will render the media useless for the intended purpose.

Glassware

Care should be taken to use clean glassware, since residues on glass may be inhibitory to some fastidious microorganisms, particularly viruses grown in cell culture, or to the cells themselves.

Quality Control

Any quality programme for culture media must in the final analysis assure that a medium will support the growth of the organisms likely to be in the specimen. It must, if specified inhibit the growth of commensal organisms, exhibit a typical biochemical response, be stable and have a reasonable shelf life. Because laboratories usually have no control over the preparation, shipping or storage of these products it is very important that they document the information that is available for each.

Physical Appearance

If the medium is stored for an excessively long time under adverse conditions or has been improperly prepared, the following signs may develop and these should be documented.

- Presence of turbidity or a precipitate indicates that some constituent has come out of the solution.
  Colours darker than normal may indicate overcooking of sugar containing media, incorrect pH or incorrect mixture of ingredients.
- Colours lighter than normal may also indicate incorrect mixture of ingredients or a wrong pH.
- Prolonged storage of medium after pouring in plates causes its dehydration and makes it unfit for use. Dehydration of the medium can be reduced by preparing only required number of plates of media and storing them by sealing plates in plastic bags.

Sterility

A few media are used without terminal sterilization, but these are exceptions; most media must be sterile when they are inoculated. Each batch of medium, whether prepared in the laboratory or received from a commercial source, should be sampled for sterility.

This is best done by removing 1-5% of the batch and placing it in a bacteriologic incubator at 35° C for 48 hours. If contaminants appear in the medium as a result of inadequate sterilization, a new lot should be obtained.

Those containers that are used for sterility testing should be discarded at the completion of the test, since they are unsuitable for inoculation because of the dehydration that occurs after up to 48 hours in the incubator.

Growth

Determine the ability of the medium to support the growth of suspected organisms by inoculating the medium with a typical stock culture isolate. A frequent quality control error is the use of a heavy inoculum for this purpose. For most media, inoculating with a stock culture that is too heavy may result in misleading growth.

In a specimen, the organism may be much more fastidious or present in very small numbers; therefore, the medium may not support its growth. When testing for the ability to support growth, it is good to prepare a dilute suspension to use as the inoculum. This suspension will give greater assurance that the medium is adequate for the growth of a small number of organisms in a patient's specimen.

In selecting an organism for testing, one should select from among the more fastidious species of organisms that one may be looking for in specimens received from patients.

Biochemical Response

When inoculating media used to identify a specific reaction, such as fermentation or H_2S production, it is necessary to use only a species or strain of organism that will produce the desired reaction.

(... To be continued)

TULIP NEWS

Tulip Group adds another feather to its cap

Striving for excellence in a global field. Emanating from a tiny state like Goa, Tulip's footprint has spread world wide. We are present on all inhabited continents. Our sun never sets.
The Tulip Group of Companies has just been accredited and awarded the highly prestigious 'CE' certification for most of its innovative *in vitro* diagnostic products.

Our Group's motto 'Quality First' has revealed the character of our products - that nothing but first class quality shall come first.
This has been recognized by the European authorities too. Tulip's products comply with the relevant European health, safety and environmental protection legislations and acts. We present the range of products that has already been awarded the coveted 'CE' mark. Very soon, all our products shall proudly display this insignia.

IMMUNOLOGY RANGE
- RHELAX - RF
- RHELAX - CRP
- RHELAX - ASO
- RHELAX - SLE

INFECTIOUS DISEASE RANGE
- CARBOGEN
- REDGEN
- TYDAL
- TYPHOCHEK
- WIDAL POSITIVE CONTROL
- BRUCEL - A
- BRUCEL - M
- BRUCEL - RB
- BRUCELLOSIS POSITIVE CONTROL
- IMMUTEX

HAEMOSTASIS RANGE
- UNIPLASTIN
- LIQUIPLASTIN
- LIQUICELIN - E
- CALCIUM CHLORIDE
- PROFACT
- FIBROSCREEN
- FIBROQUANT
- TULIP XL- FDP
- PLASMATROL H I / HII

IMMUNOHAEMATOLOGY RANGE
- ERYBANK ANTI- A1 LECTIN
- ERYBANK ANTI- H LECTIN

IMMUNOTURBIDIMETRY RANGE
- QUANTIA RF
- QUANTIA ASO
- QUANTIA CRP UV
- QUANTIA CRP-US
- QUANTIA MA
- QUANTIA IgG
- QUANTIA IgM
- QUANTIA IgA
- QUANTIA C3
- QUANTIA C4
- QUANTIA AT III
- QUANTIA Lp(a)
- QUANTIA Fibrinogen
- QUANTIA Apo-A1
- QUANTIA Apo-B
- SEROQUANT RF(Level1&2)
- SEROQUANT ASO (Level1&2)
- SEROQUANT CRP (Level1&2)
- SEROQUANT CRP-US(Level1&2)
- SEROQUANT MA (Level1&2)
- SEROQUANT Lp (a) (Level1&2)
- SEROQUANT APO- A1(Level1&2)
- SEROQUANT APO- B (Level1&2)
- SEROQUANT FIBRINOGEN (Level1&2)
- SEROQUANT AT III (Level1&2)
- SEROQUANT IgG (Level1&2)
- SEROQUANT IgM (Level1&2)
- SEROQUANT IgA (Level1&2)
- SEROQUANT C3 (Level1&2)
- SEROQUANT C4 (Level1&2)

RAPID TESTS
- RETROCHECK HIV(Device)
- SYPHICHECK -WB (Device)
- GRAVICHECK(Device)
- GRAVICHECK(Dipstick)
- GRAVISCREEN(Device)
- GRAVISCREEN(Dipstick)
- CLUE(Dipstick)
- CLUE(Device)
- PARACHECK Pf(Dipstick)
- PARACHECK Pf(Device)
- DENGUCHECK -WB(Device)
- LEPTOCHECK- WB(Device)
- SEROCHECK -MTB (Device)
- ENTEROCHECK- WB (Device)
- FALCIVAX(Device)
- PARASCREEN(Device)
- PARAMAX - (Device)
- PARABANK(Device)
- PARABANK (Dipstick)
- AMICHECK TROP I WB(Device)

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Quantia and Quantiamate

Cardiovascular Risk Marker Range

Immunoturbidimetry... ...Now, Take Complete Control

Quantia Reagents

PRODUCT	APPLICATION	PRESENTATION
QUANTIA - CRP-US	Quantitation of ultrasensitive levels of C-reactive protein	50 Tests
QUANTIA - Lp (a)	Quantitation of Lipoprotein (a)	50 Tests
QUANTIA - APO A-1	Quantitation of Apolipoprotein A-1	50 Tests
QUANTIA - APO B	Quantitation of Apolipoprotein B	50 Tests
QUANTIA - FIBRINOGEN	Quantitation of Fibrinogen	25 Tests

Printed and published by D.G. Tripathi, Edited by Dr. R.J. Sood M.D. (path.) for and on behalf of Tulip Diagnostics Private Ltd, Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh Alto Santacruz, Bambolim Complex Post Office, Goa - 403202, INDIA. Fax: (0832) 2458544, E-mail:sales@tulipgroup.com Website: www.tulipgroup.com