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The Crux

BIMONTHLY FORUM FOR THE LABORATARIANS

CONTENTS

Editorial

As a forum like this can not possibly present the stability calendar of all analytes of relevance to human beings in a single issue, the topic is continued from previous issue and few of the other commonly tested for analytes are covered in this communication. This section is concluded in this copy. You are most welcome to put your requests for stability calendar of analytes of importance to you. All possible detailed information shall be relayed to you. The TROUBLE SHOOTING segment is a continuation from the last communiqué.

DISEASE DIAGNOSIS discusses Enteric fever this time. The disease is still rampant in the third world. It causes significant morbidity and has a case fatality rate varying between 1.1 to 2.5 %. It is an important communicable disease and is found only in man. A disease of poor environmental sanitation and unsafe water supply, typhoid spreads by the feco-oral route. The disease can be diagnosed and treated easily. A proper diagnosis induces early and correct therapy that in turn reduces both, the morbidity and mortality and also assists in arresting further spread of the disease. Newer diagnostic platforms are now available that are not so cumbersome and can be used by the bedside too. These immunological device tests detect IgM antibodies to the S. typhi "O" antigen. The utility of these tests is that they indicate presence or absence of an ongoing current infection. In times to come, probably, we shall be enabled to give confirmed bedside diagnosis for many diseases.

An investigation that has acquired a parallel and perhaps greater diagnostic (non specific, though) and prognostic significance than ESR is C-Reactive Protein. Interpreted in the light of clinical background, CRP values can provide extremely useful diagnostic information. An enhanced CRP is always associated with pathological changes. Therefore, determination of CRP is of value in diagnosis, treatment and monitoring of inflammatory conditions. Pediatric fever is an example that can be cited. Pediatric febrile episodes (upper respiratory tract infections) are usually caused by viruses and antibiotics are prescribed routinely where they have no therapeutic role whatsoever. It has been shown that, in children who have been ill for more than 12 hours, a CRP level of greater than 4 mg/dl has a diagnostic sensitivity of 79%, and a specificity of 90% for the diagnosis of bacterial infections. This simple investigation can obviate the unnecessary use of antibiotics. INTERPRETATION portion of this issue thrashes out the effective use of CRP as an acute phase reactant While ESR has been used for decades as a non-specific test to detect and monitor the presence of inflammatory activity in the body. It has also been known that factors like albumin/globulin ratio, degree of anemia (if present) can significantly alter the ESR reading. Estimation and use of CRP as an acute phase reactant does not suffer from these drawbacks. If base level of CRP of an individual were known, a slight increase in its value would indicate the onset of an inflammatory lesion somewhere in the body. Much, much before the patient becomes symptomatic the CRP levels would exhibit an upward trend.

BOUQUET has all the usual ingredients. Check if the WISDOM WHISPERS or shouts this time? Which sub-speciality of pathology has overwhelmed the BRAIN TEASERS? And LIGHTER VEIN has become heavy with laughter loaded in every joke.

ISO 9001

Tulip Group

DISEASE · DIAGNOSIS

ENTERIC FEVER (Synonym=Typhoid Fever)

A systemic communicable disease of MAN caused by *Salmonella typhi* organisms. They cause an acute generalized infection of the reticulo-endothelial (RE) system, intestinal lymphoid tissue, and the gall bladder. Established in late 19th century as a distinct clinical entity, enteric fever encompasses both, typhoid and paratyphoid fevers. Unsafe water supply, poor environmental sanitation are held responsible for spread. Incidence in India is about 7.6 per 1,000 per year. Man appears to be the only host, reservoir, and chronic carrier.

Important Epidemiological Features

Age Group: Any age, but predominantly children and young adults are involved. Highest attack rate occurs between 8-13 years.

Gender and Race: No racial affinity. Males on account of greater mobility are infected more, while females have a special predilection for becoming chronic carriers. Remember TYPHOID MARY!

Occupation: Those handling the infective material and live cultures of *S. typhi* are at increased risk.

Socio-economic factors: It is a disease of poverty and is often associated with inadequate sanitation facilities and unsafe water supplies.

Nutritional status: No direct evidence links the two but malnutrition may enhance susceptibility to typhoid fever by altering the intestinal flora or other host defenses.

Environmental factors: Peak incidence is during July - September in India. This being the rainy season with substantial increase in fly population. Typhoid bacilli are commonly found in water, ice, food, milk, and soil. They do not multiply in water but can survive in ice and ice creams for up to a month and up to 70 days in soil irrigated with sewage.

Reservoir of infection: Man is the only known reservoir of infection cases or carriers. Propagation of the disease occurs through urine or stool. Carriers may be temporary or chronic. Temporary (convalescent or incubatory) carriers usually excrete bacilli up to 6-8 weeks. By the end of one year, 3-4 percent of cases continue to excrete typhoid bacilli (chronic carriers). Fecal carriers are more frequent than urinary carriers. Carriers may shed bacteria continuously or intermittently.

Clinical Features

Incubation period varies from 3 to 30 days and depends upon inoculum size and host defense. Classical onset of the disease is daily remittent fever pattern with temperature variation between 40 to 41 degrees Celsius, which is usually associated with chills, headache and malaise. Early intestinal manifestations may be constipation and diarrhoea, the latter being commoner in children with abdominal tenderness. An untreated case may have prolonged fever, which may become persistent.

Frequency Of Symptoms In Typhoid Fever (in decreasing order of frequency)

Typhoid fever: Fever, headache, constipation, diarrhoea, abdominal cramps, cough, nausea, vomiting Paratyphoid A & B: Fever, headache, abdominal cramps, diarrhoea, cough, nausea, vomiting, constipation. Few of the cases may have nonspecific symptoms like cough and conjunctivitis. Illness may be mild and short for some, however, it may manifest as an acute severe infection associated with disseminated intravascular coagulation and may also involve the CNS leading to early mortality. Other severe manifestations include necrotising cholecystitis, intestinal bleeding, and perforation; which may be sudden and during recovery phase. Intestinal perforation is less common in very young children. Infection caused by Salmonellae other than *S. typhi* is usually less severe and for shorter duration.

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Physical Findings (in decreasing order of frequency)

Typhoid fever: Fever, abdominal tenderness, rales or rhonchi, splenomegaly, hepatomegaly, relative bradycardia, rose spot in the umbilical region, epistaxis, meningism.

Paratyphoid A & B: Fever, relative bradycardia, rales or rhonchi, splenomegaly, hepatomegaly, abdominal tenderness, epistaxis, meningism, rose spots in the umbilical region.

Fever is the commonest symptom and finding, however, afebrile individuals may be culture positive.The temperature graph in typhoid fever may be characteristic The fever is remittent in the first week, rising in a stepwise fashion and may become persistent in the later phase of the illness. Deviations in this pattern are known to occur. In the early phase the only physical findings may be pyrexia, coated tongue and relative bradycardia in a few patients. Hypotension suggests severe disease with septicemia. Prominent respiratory signs with a normal skiagram suggest typhoid fever. Abdomen may show diffuse tenderness without guarding and a moderate degree of splenomegaly. Soft tender hepatomegaly is usually present by the second week of illness. The third week brings up a typical typhoid face appearance, where the face looks thin, pale, with wide bright eyes with apathetic staring expression. Depending upon system and severity of involvement, other physical findings may include tremor, gait ataxia, cardiac signs, jaundice etc.

The important features of untreated typhoid fever are high fever and anorexia that is associated with a change of sensorium. A number of systemic complications have been reported, which includes hepatitis, meningitis, nephritis, myocarditis, bronchitis, pneumonia, arthritis, osteomyelitis, parotitis, and orchitis. Relapse of illness, usually in inadequately treated patients, is quite frequent. Drug resistant *S. typhi* infection is becoming more common in endemic countries. About 3-5% patients may become chronic asymptomatic carriers.

Parathyphoid Fever

These infections are milder than typhoid fever.

Salmonella paratyphi A

S. paratyphi A tends to produce an illness clinically akin to that produced by *S. typhi* with prolonged fever and a tendency to relapse. This is the commonest paratyphoid fever in India as well as rest the Asia.

Salmonella paratyphi B (Salmonella schottmulleri)

S. paratyphi B outbreaks are more frequently food-borne than water-borne and carrier state is more common. *Paratyphi B* is milder than typhoid but is more prone to cause jaundice, suppurative lesion, and carrier state. Ulceration can occur in stomach and large intestine as well. This serovariant is rarely reported from India.

Salmonella paratyphi C (Salmonella hirschfeldii)

Infection with this organism has been reported from Eastern Europe, India, Guyana, and Central and East Africa.

The clinical course in young children is little different than in the adults. In children it may present as an acute febrile illness associated with symptoms like diarrhoea, vomiting, and sometimes predominant respiratory symptoms; often diagnosed as gastroenteritis or respiratory tract infection. Complications like meningitis, convulsions, and jaundice are more frequent and are usually associated with mortality. The *differential diagnosis* of typhoid includes conditions as: malaria, brucellosis, viral hepatitis, kala-azar, infectious mononucleosis, amebic liver abscess, meningitis, rheumatic fever, endocarditis, focal abscesses, septicemia (due to other systemic infections), miliary tuberculosis, and several other viral fevers.

Typhoid is a multisystem disease, untreated, it can cause prolonged illness, systemic complications and high mortality. Intestinal haemorrhage occurs in about 5% (0.5 to 10%) of the cases, usually during the second or third week of the illness, even when the patient is on antibiotic therapy. Intestinal perforation is a serious complication and may occur in 1% of the cases. Perforation of the small bowel is a surgical emergency.

Acute cholecystitis is also a fairly common complication, however, classical symptom of acute cholecystitis may be absent.Typhoid may increase the incidence of gallstones.

Jaundice is reported in 1% cases of typhoid fever and is called as typhoid hepatitis (hepatomegaly with raised transaminases), septicemia, or liver abscesses. An increase in neuropsychiatric complications has been found in the last few years. In some cases they may dominate the clinical picture. Other systemic complications are listed below.

Systemic complications

System	Complications
Gastrointestinal	Intestinal hemorrhage and perforation, acute cholecystitis, acute pancreatitis hepatic abscess, splenic rupture, typhoid hepatitis.
Neuropsychiatric	Delirium, depression, psychosis, meningitis, encephalopathy, optic neuritis.
Respiratory	Bronchitis, pneumonia, pleural effusion, pneumothorax
Hemopoeitic	Hemolysis, DIC
Cardiovascular	Myocarditis, pericarditis, endocarditis, shock
Genito-urinary	Glomerulonephritis, pyelonephritis, cystitis
Skeletomuscular	Periostitis, typhoid spine, muscular rupture
Others	Bed sores, hypercalcemia, decubitus ulceration, abortion, etc.

Relapse of typhoid fever may occur in some patients (10-20%), several weeks later or after apparent recovery. There may be days or weeks between the two attacks and usually, the relapse is milder than the primary attack.

Pathogenesis

As per the new nomenclature all Salmonellae that cause enteric fever in human are grouped and named *Salmonella enterica* and the previous species names are assigned as serotypes eg., *S enterica* serotype Typhi, Paratyphi A, B, C, etc. Salmonellae penetrate the mucosa of both small and large bowel and proliferate within these cells. Shigellae cause greater degree of mucosal damage but Salmonellae cause ulceration of lymphoid follicles. Initially *S typhi* proliferates in the second part of the Payer's patches of the lower small intestine from where systemic dissemination occurs to the liver, spleen, and reticuloendothelial system. For a period varying from 1 to 3 weeks the organism multiplies within these organs.

Rupture of infected cell occurs, liberating organisms into the bile and for a second time cause infection of the lymphoid tissue of the small intestine particularly in the ileum. It is this phase of heavy infection that brings the classical bowel pathology of typhoid in its train. Invasion of the mucosa causes the epithelial cells to synthesize and release various proinflammatory cytokines including IL-1, IL-6, IL-8, TNF-ß, INF, GM-CSF etc.

Pathology

Pathology in the Payer's patches has been assumed to be occurring in four phases. These phases correspond approximately to the weeks of disease if treatment has not been given.
Phase 1: Hyperplasia of lymphoid follicles.
Phase 2: Necrosis of lymphoid follicles during the second week involving both mucosa and submucosa.
Phase 3: Ulceration in the long axis of the bowel with the possibility of perforation and hemorrhage.
Phase 4: Healing takes place from the fourth week onward, and unlike tuberculosis of the bowel with its encircling ulcers,does not produce strictures.

Although the ileum is the classical seat of typhoid pathology, lymphoid follicles may be affected in parts of the gastrointestinal tract, such as the jejunum and ascending colon. The ileum usually contains larger and more numerous Payers' patches than the jejunum, but this is not an invariable finding. It is not generally appreciated that such lymphoid follicles are also found in the large intestine. The number of solitary follicles in large intestine decreases with age. Ulceration during paratyphoid B infection may involve stomach and large intestine as well. The typhoid perforations as usually being simple and involving the antimesenteric border of the bowel where they appear as punched out holes. In contrast to other types of perforation omental migration to the affected area does not occur.

The reticuloendothelial system, enlargement and congestion of the spleen and mesenteric glands are characteristic finding.The so-called typhoid hepatitis has been described when a liver biopsy may show non-specific reactive hepatitis. The salient features on liver biopsy are focal liver cell necrosis with associated infiltration of mononuclears - typhoid nodules - sinusoidal congestion and dilation, and mononuclear cell infiltration of the portal area. Hepatitis should not be forgotten as one of the complications of typhoid and paratyphoid fever.

Laboratory diagnosis

The presence of *S.typhi* or *S.paratyphi* is detected either by culture of the organism or by demonstration of specific antibodies or antigen in the serum or urine. The organism may be cultured from blood, bone marrow, stool or urine.

Culture

In addition to the usual two bottles inoculated with blood, a third bottle containing streptokinase bile salt broth can significantly increase the isolation rate of *S typhi*. Although the conventional wisdom is that *S typhi* is obtained from blood during the first week of illness more frequently than from the stool, whereas the reverse applies during the second and third weeks of the illness, the clinician should be reminded that the organism can be cultured from blood as late as the fifth week of the disease, and the organism may be cultured from the stool throughout the disease. The organism is less frequently isolated from urine, but it is useful to determine whether a patient does excrete the organism in the urine because this could become a site for chronic carriage. Culture of bone marrow or skin snips taken from rose spots may yield the organism when it cannot be obtained from blood, stool, or urine. The organism can be cultured from the bone marrow in as many as 96 percent of patients even after antibiotics have already been given.

The liquid and solid media that are suitable for isolation of *Salmonella typhi* and salmonellosis are several. However, strontium selenite broth is superior to selenite F broth for the isolation of *S.typhi* especially when relatively few typhoid bacilli are present in feces, for example after antibiotic therapy or if stool specimens have been left for prolonged periods at room temperature; and salmonella - shigella agar has been found to be superior to xylose lysine deoxycholate agar for the isolation of *S. typhi*. Modified bismuth sulphate agar is superior to deoxycholate agar for the growth of Salmonella sp. and is mandatory

if the diagnosis of typhoid is very likely, or if a carrier is being investigated. Automation in bacteriology has simplified the bacteriologists task and has reduced the total reporting time. The Salmonella culture can become positive as early as 4 hours after blood sampling.

In conclusion, bone marrow is the gold standard for culturing the organism. It can yield positive results even if the patient has started antibiotics. Although blood culture is most likely to yield the organism during the first and third week, or septicemic phases of the illness, the clinician is advised to order blood, stool, and urine cultures on one or more occasions to confirm or exclude the diagnosis.

Serological diagnosis

Antibody detection

The Widal test has long been used as a serological aid in the diagnosis of typhoid fever. Two specimens of serum are required at an interval of 7-10 days and a four-fold rise in the titers of H (flagellar) or O (somatic) agglutinins indicates a strong likelihood of the disease. Previous TAB immunizations may leave residual titers of H agglutinins for years, and a rise in O agglutinins may be more relevant in such patients. However, even in immunized patients it is possible to get a rise only in H agglutinins and not in O agglutinins. The Widal test has the disadvantage that diagnosis is delayed until a second specimen is received. The Widal test can be performed on a single serum, different geographic locations keep different cut off titers. Most Widal test kits suffer from an inherent problem in that the suspension fluid of the bacteria bears the same colour as the bacteria themselves. This can sometimes create reading/ interpretation problems. A kit that provides a clear suspension medium for the stained salmonellae would obviate this problem and make the laboratarians job that much simpler.

Now ELISA and Immunochromatographic formats are also available which have increased specificity and sensitivity of the diagnosis. The levels of both IgM and IgG antibodies can be ascertained.

Antigen detection

Counter-immunoelectrophoresis (CIE) of a single specimen of serum to detect *S. typhi* O antigen can yield a positive result early in the disease. Rapid latex agglutination test has also been developed to detect specific antigens in the culture supernatants. Its main utility is in rapid identification of species of Salmonella.

Salmonella typhi has also a Vi antigen, and antibodies to this antigen can be looked for in a patient's blood, but it has historically been used to diagnose a chronic carrier of *S. typhi*. *S. typhi* can be subdivided for useful epidemiological purposes by phage typing; there are 80 Vi phage types. Phage typing is required to establish identity of strain between source and patient.

Diagnosis of typhoid carriers

Carriers of *S typhi* are either convalescent carriers who excrete the organism for a limited period of time after apparent clinical cure, or chronic carriers in whom persistent excretion of *S typhi* in stool or urine can be detected a year after clinical illness. Chronic fecal carriers occur more commonly than do chronic urinary ones. The numbers of typhoid bacilli excreted in the stools of these cases may be inordinately large, each gram of feces usually containing 10 or more viable organisms. The diagnosis of carrier status is established by culturing the organism from the relevant specimen of the suspected person.

Gelatin capsule string test is preferable for detection of chronic fecal carrier. Because excretion of organisms in the feces of chronic carriers is often intermittent, methods other than fecal cultures have been devised to increase the sensitivity of culture. One such method is to culture the duodenal aspirates in suspected gall bladder carriers.

Vi-antibody tests

Serological tests are used to screen people suspected of being chronic carriers of *Salmonella typhi.* The Vi agglutination test has been used for many years.

BOUQUET

IN LIGHTER VEIN

•A drunkard was coming home from a local liquor shop late at night. He lived alone and locked his house whenever he went out . As he neared his house he took out his key to open the lock, but he could not manage to put the key into hole. After trying repeatedly, he was tired. A neighbour who was witnessing the scene took pity on him and said, “Give me the key I will get it open for you.” The drunkard looked for a while, and said to him, “The lock will be opened by me, but do me a favour, please hold the house firmly, while I do the rest. Damn it, it is shaking like a pendulum.”

•A Managing Director was interviewing a charming lady for the post of Personal Secretary. Finally he asked the lady what salary she expected? Very modestly she replied “Rs. 2500, Sir.” “with pleasure,” said the Managing Director. “In that case Rs. 3500,Sir,” was the prompt reply by the lady.

WISDOM WHISPERS

- He who looses wealth looses much;
  He who looses a friend looses more
  He who looses courage looses all.
- God grant me the serenity to accept the things I cannot change… courage to change the things I can… and the wisdom to know the difference!
- Think of the customer first if you would like to have the customer think of you first.
- Hold fast to dreams, for, if dreams die,
  Life is a broken winged bird that can not fly.
- The shortest way to do many things is to do only one thing at a time.
- If it falls your lot to be a street sweeper; sweep the streets like Michelangelo painted pictures, like Shakespeare wrote poetry, like Beethoven composed music, sweep streets so well that all the hosts of heaven and earth will have to pause and say “Here lived a great street sweeper; who swept his job well.”
- Failure is success if we want to learn from it.
- Never fight a man who has nothing to lose.
- Opportunity …. Often comes disguised in the form of misfortune, or temporary defeat.

BRAIN TEASERS

1. Russel bodies are seen:
   A) Plasma cells B) Kupffer cells C) Neurons D) Mast cells.
2. Cholesterolosis occurs in:
   A) Aorta B) Gall bladder C) Heart D) Cholesteatoma.
3. Which tumour can cause episodes of hypoglycemia?
   A) Retroperitoneal fibroma B) Haemangioma C) Meningioma D) Lymphangioma.
4. Pleural effusions can be associated with which of the following?
   A) Neurofibroma B) Lipofibroma C) Dermatofibroma D) Ovarian fibroma.
5. Vitamin B_{12} is absorbed from:
   A) Stomach B) Duodenum and jejunum C) Terminal ileum D) Cecum
6. Crypt abscess is seen in:
   A) Ulcerative colitis B) Non specific colitis C) Amoebic colitis D) Staphylococcal colitis
7. Schaumann's body is found in:
   A) Actinomycosos B) Sarcoidosis C) Asbestosis D) Berryliosis
8. Donovan body is seen in:
   A) Granuloma inguinale B) Chancre C) Condylomata lata D) Chancroid

Answers: 1 - A, 2 - B, 3 - A, 4 - D, 5 - C, 6 - A, 7 - B, 8 - A.

TROUBLE SHOOTING

It is beyond the scope of a forum like this to present stability calendar of all known analytes of importance and relevance to human beings. An effort was made in the last communiqué towards this direction. The remaining commonly tested for analytes are covered in this issue and are presented below.

Analyte	Stability in Serum / Plasma			Stabilizer	Analytical Levels Affected by
	- 20 °C	4 - 8 °C	20 - 25 °C		
Lead	?	?	7 days		Special tubes
Lipase	1 year	7 days	7 days		heat exposure,EDTA, hemolysis, bilirubin
Lipoprotein (a) - Lp(a)	3 months	2 weeks	?		Affected by lipemia, turbidity
Lutropin (LH)	1 year	3 days	1 day		Affected by heat exposure, EDTA, HAMA
Magnesium	1 year	7 days	7 days		Affected by hair treatments
Myoglobin	3 months	1 day	2 hours		Affected by EDTA, lipemia
Neuron-specific enolase	3 months	3 days	?	Heparin Plasma	Serum > plasma (Platelets, hemolysis)
Osmolarity	3 months	1 day	3 hours		Gycolysis, citrate
Osteocalcin	Stabilized 14 days	?	Unstable	EDTA (5 mmol/l) and aprotinin (2500 KU/ml)	Bilirubin, citrate, EDTA, Freeze-thaw cycle, hemolysis
Parathyroid hormone (PTH)	?	1 day	6 hours	EDTA	Delayin freezing, separation of cells
Phosphate, inorganic	1 year	4 days	1 day		Bilirubin, fluoride, lipemia
Potassium	1 year	1 week	1 week		Serum>plasma
Progesterone	1 year	3 days	1 day		Blood collection tubes, Cross reactivity with hydroxyprogesterone & lysophosphatidylcholine
Prolactin	1 year	3 days	1 day		Bilirubin, heat exposure Cross reaction with Growth hormone
Prostatic-specific antigen (PSA)	3 months	2 days	1 day		Affected by rectal examination, transrectal biopsy
Protein electrophoresis	3 weeks	3 days	1 day		
Protein, total	Years	4 weeks	6 days		Plasma> serum (fibrinogen)
Rheumatoid factor (RF)	4 weeks	3 days	1 day		
Selenium	1 year	2 weeks	1 week		Contamination
Sodium	1 year	2 weeks	2 weeks		Bilirubin, heparin, lipemia, pH
Testosterone	1 year	3 days	1 day		SHBG, Androstenedione, DHT, Heat exposure, delay in separation of cells
Thyroglobulin	4 weeks	3 days	1 day		
Thyrotropin (TSH)	3 months	3 days	1 day		Alkaline phosphatase, EDTA, HAMA Freeze-thaw cycles, heat exposure
Thyroxine (T4)	4 weeks	7 days	2 days		Freeze-thaw cycles, Citrate, hemolysis, oxalate
Transferrin	6 months	8 days	8 days		Bilirubin, Urea, heat exposure
Triglycerides	Years	7 days	2 days		Decrease of triglycerides, of ↑free glycerol, but only minor ↑of total glycerol
Triiodothyronine (T3)	3 months	8 days	2 days		Freeze-thaw cycles, Citrate, protein, heat exposure
Troponin T	3 months	1 day	?		Citrate, heparin, EDTA
Urea	1 year	7 days	7 days		
Uric acid	6 months	7 days	3 days		Bilirubin, cyanide, EDTA, formaldehyde, hemolysis, lipemia, fluoride
Vitamin A	2 years	4 weeks	?		Light (decrease)
Vitamin B1 (thiamin)	1 year	?	?		
Vitamin B2 (riboflavin)	4 weeks	?	?		Light (decrease)
Vitamin B6 (pyridoxal phosphate)	Days	Hours	30 minutes	EDTA plasma darkness	Light (decrease)
Vitamin B	128 weeks	4 hours	15 minutes	EDTA, Darkness	Light (decrease)
Vitamin C	3 weeks	3 hours	?	Metaphosphate 63 mg/ml deproteinized	
	Only with stabilizers				
Vitamin D - 1,25 Dithydroxy cholecalciferol	?	?	2 days		
Vitamin E (tocopherol)	1 year	4 weeks			
Vitamin K (transphyllochinone)	3 months	Unstable	?		UV Light (decrease), use extraction
Zinc	1 year	2 weeks	1 week	Special tubes	Contamination from stoppers

INTERPRETATION

C-REACTIVE PROTEIN

CRP is an abnormal serum glycoprotein produced by the liver during acute inflammation or infections. CRP is synthesized by the liver under regulatory control of cytokines. Interleukins 1b and 6 and tumour necrosis factors are the most important regulators of CRP synthesis.The intact CRP molecule is a pentameric protein with identical sub unit arranged in a doughnut shaped polymer.

The function of CRP is felt to be related to its role in the innate immune system. Similar to IgG it activates complement, binds to Fc receptor and acts as an opsonin for various pathogens. Interaction of CRP with Fc receptors leads to the generation of proinflammatory cytokines that enhance inflammatory response. Unlike IgG, which specifically recognize distinct antigenic epitopes, **CRP recognizes altered self and foreign molecules based on pattern of recognition.** This recognition provides an early defense and leads to a proinflammatory signal and activation of the humoral immune response. CRP binds to apoptotic cells, protects the cells from assembly of terminal complement components and sustains an antiflammatory innate immune response.

All acute inflammatory process (infectious and non-infectious) and certain malignant conditions result in rise in serum CRP, as a non-specific phenomenon. CRP production is a non-specific response to disease and it can never on its own be used as a diagnostic test. **However if the CRP results are interpreted in the light of clinical information on the patient it can provide exceptionally useful information.**

Levels of CRP increase very rapidly in response to trauma, inflammation and infection and decrease very rapidly with the resolution of the condition. **An activated CRP is always associated with pathological changes.** Hence **determination of CRP is of great value in diagnosis, treatment and monitoring of inflammatory condition.** Measurement of CRP may be helpful to know whether the patient is getting better, or if there are any complications arising.

CRP measurements help in diagnosis and management of: -

Rheumatology	The rheumatic diseases exhibit joint or soft tissue symptoms such as back pain, and myalgia. However such symptoms may also be due to psychogenic factors. The elevation of acute phase proteins confirms the presence of organic disease but a value within the reference range does not exclude a mild local disease. In conditions like ankylosing spondylitis, serum CRP may be elevated before the onset of clinical symptoms.
Adult rheumatoid Arthritis	Increased CRP levels are found in more than 90% of adults with this condition and in established disease, levels relate to severity. Values of up to 5 mg/dl are associated with mild inflammation and values around 10 mg/dl indicate more severe disease. Certainly CRP levels correlate more closely with radiologically determined joint damage than other serological tests. Typically if a patient responds to a particular drug the fall in CRP precedes the improvement in clinical symptoms by about 6 weeks and the radiological improvement by about 6 months.
Ankylosing Spondylitis	Back pain is a very common clinical symptom and an elevated CRP is a strong indication of organic disease such as ankylosing spondylitis.
Polymyalgia Rheumatica	CRP concentration is markedly raised. If untreated 30% of patients will develop cranial arteritis with a serious risk of eyesight. CRP rapidly falls to normal as the disease responds to therapy with corticosteroids.
Connective tissue Diseases	SLE, polymyositis, systemic sclerosis, in these cases acute phase response is minimal even in active disease. Hence CRP levels can be used to distinguish these conditions from other rheumatic diseases.
Infections	Bacterial infections are associated with some of the highest CRP levels and its measurement is a sensitive marker for bacterial sepsis. Gram negative bacteria generally elicit more reproducible responses than gram positive bacteria, with modest responses to parasitic infestations and minor responses to viruses and fungi. CRP measurement is useful in detecting infections where clinical and microbiological diagnosis is difficult but where infection is suspected. CRP levels relate to the extent and intensity of sepsis and successful treatment leads to decline in levels within about three days.
Pediatric Fever	**In children, although fever is most often due to viral infection, this is difficult to distinguish from bacterial sepsis such as otitis media, bronchitis, tonsillitis, and cystitis, and antibiotics are often prescribed unnecessarily**. It has been shown that, in children who have been ill for more than 12 hrs, a CRP level of greater than 4 mg/dl had a diagnostic sensitivity of 79%, and a specificity of 90% for the diagnosis of bacterial infections.
Adults post operative Surgery	Surgery of all types induces inflammation and an acute phase response roughly in proportion to the extent of tissue damage. In uncomplicated cases CRP rises above 1 mg/dl by about 6 hr., reaches a peak rarely greater than 15 mg/dl at about 48 hrs., and declines thereafter to baseline values by 7- 10 days . Postoperative complications such as infections, tissue necrosis, hematoma , and thromboses, depending upon when they occur, will maintain a raised CRP level after 48 hrs., or result in a secondary increase. In many cases the raised CRP precedes the clinical diagnosis of the complicating pathology by upto 24 hrs. In such situations single values are of little value and serial monitoring is essential.
Appendicitis	Using a cut off of 1 mg/dl it has been reported that CRP has clinical sensitivity for this condition of 68.2% and a specificity of 75.1%.
Meningitis	Some studies using serum CRP have described almost perfect discrimination between bacterial and viral meningitis in children. Bacterial meningitis is associated with higher CRP levels than aseptic or viral meningitis. Appropriate therapy for bacterial and tuberculous meningitis causes fall in CRP levels, and **hence this simple test can be used to monitor response to treatment with many advantages over repeated lumbar punctures especially in children.**
Pulmonary infection	Pneumonia can be difficult to diagnose in the elderly where the febrile response may be lost. A CRP level above 10 mg/dl provides a very strong indication of bacterial infection such as purulent bronchitis or pneumonia. Typically viral pneumonia's do not result in values above 5 mg/dl.

Malignant tumors	Increasing levels of CRP imply a poor prognosis and frequently suggest metastatic spread.
Burns	CRP levels increase significantly in patients with extensive burns. A second peak of CRP later implies superadded infection as a late complication of burns.
Myocardial infarctions	Peak CRP levels occur about 50 hrs after the onset of pain in myocardial infarction, and correlate well with peak serum levels of cardiac isoenzymes such as CKMB. In patients who recover uneventfully the CRP levels fall rapidly towards normal. However complications such as persistent cardiac dysfunction further infarction, intercurrent infection, thromboembolism, are associated with either persistently raised CRP levels or secondary increase after initial decrease. Angina without infarction does not stimulate CRP production. Routine assays of CRP in patients with chest pain may thus assist in diagnosis, and management of complications.
Immunocompromised patients in Acute leukemia.	Fever in patients with leukemia and neutropenia can be caused by infection, the underlying disease process, administration of blood products, and cytotoxic therapy. Approximately 40% of cancer patients with fever and neutropenia develop culture proven bacterial infections. Fever can also be caused by viral infections or maybe by other non-infectious causes. Because of significant morbidity and mortality in this group, there is aggressive use of antibiotics. Chemotherapy or transfusions do not affect CRP. Pronounced elevations of CRP do not occur in malignancies without other concomitant stimuli for synthesis such as intercurrent infections. If CRP concentration is less than 4 mg/dl for 48 hrs after the onset of fever, infection is unlikely, whereas levels above 10 mg/dl should be treated by antibiotics even in absence of bacteriological confirmation. If after treatment levels do not fall below then it must be assumed that response has not occurred and therapy must be maintained and changed.

It is often difficult to diagnose abdominal infection in pregnant women, since CRP is at normal levels in pregnant women, increased CRP concentration indicates infection complication.

Bacterial sepsis is one of the most common diagnostic challenges in neonatal medicine. A definitive diagnosis based on culture of blood, CSF or urine is usually reached only after a delay of a day or two, yet rapid progression of untreated infection may greatly increase morbidity and mortality. Initiation of antibiotic therapy may result in treatment of as many as 30 uninfected infants for every single infant who is determined to have been infected. Attempts to develop a screening test that can identify infected infants, sparing others from invasive diagnostic procedures, intravenous antibiotic therapy, mother infant separation and heightened parental anxiety has led to the observation that CRP levels during these intervals may be useful for early identification of infants for whom antibiotic therapy can be safely discontinued. In addition to better management of disease or disorders, CRP has been known to aid in the differential diagnosis of many illnesses.

ROLE OF CRP IN DIFFERENTIAL DIAGNOSIS

Clinical Condition	Significantly Elevated CRP	Normal CRP/ Mildly elevated CRP
Rheumatic diseases	In established RA disease- levels relate to severity. Values upto 5 mg/dl are associated with mild inflammation and values around 10 mg/dl indicate more severe disease.	Normal in Osteoarthritis.
Gastrointestinal diseases (inflammatory bowel disease)	Crohn's disease	Ulcerative colitis, normal CRP or mild elevation < 5 mg/dl
Pediatric fever	Children ill for more than 12 hr. with CRP > 4mg/dl generally indicates bacterial infection	CRP level < 4 mg/dl may be bacterial or viral infection
Genital infections	Chlamydial infections when extended into the pelvic organs with acute or chronic pelvic inflammatory disease	Uncomplicated gonococcal or chalmydial infection Not elevated
Pulmonary infection	Above 10 mg/dl provide a strong indication of bacterial infection such as pneumonia or purulent bronchitis	Typically viral pneumonia does not result in values above 5 mg/dl
Causes related with chest pain	Elevated in pulmonary embolism, pleurisy, or pericarditis	Not elevated in angina without infarction or invasive investigation
UTI in young children	Values > 5 mg/dl indicate pyelonephritis	Normal to slightly elevated levels indicates uncomplicated UTI

The degree of elevation of CRP reflects the mass or activity of the inflammed tissue, which may be secondary to the underlying disease as in myocardial infarction and malignancy, or a primary component as in rheumatoid arthritis.

In many cases the changes in plasma CRP levels precede changes in clinical symptoms. In every situation sequential measurements provide more information than single determinations.

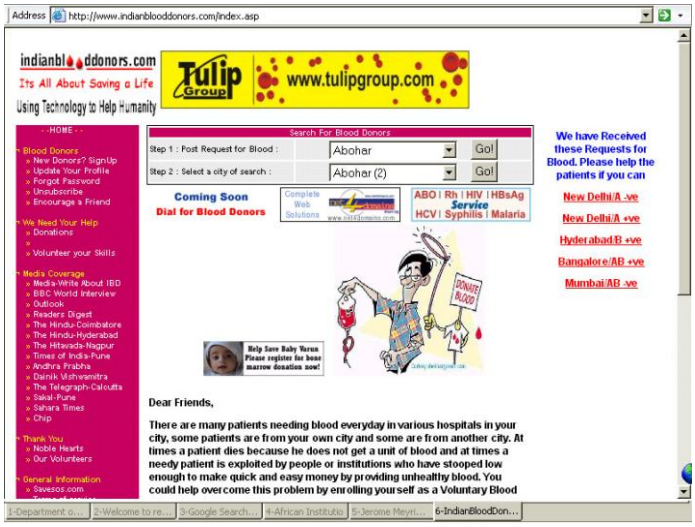
To summarize, Quantitative CRP measurement would be useful in

- Screening of organic diseases
- Differential diagnosis
- Assessment of disease activity and monitoring of therapy
- Recognition of intercurrent infections
- Prognosis of conditions such as myocardial infarction

TULIP NEWS

Since its inception, TULIP GROUP has many "firsts" in terms of introduction of novel technology, products and processes in the market. Another "first" from TULIP GROUP is particularly in the Blood Banking segment.

Apart from being a world-class in-vitro diagnostic company, TULIP is also committed to a social cause like VOLUNTARY BLOOD DONATION. TULIP believes that awareness in voluntary blood donation in the society is critical. A precious life productive to the society may be lost due to lack of just a single unit of blood! Like-minded individuals and organizations can stop this loss to society with coordinated efforts.



In this endeavor, TULIP Group is pleased to be the co-sponsor of www.indianblooddonors.com. www.indianblooddonors.com is a website dedicated to saving lives. It offers service to enhance access to voluntary blood donation in times of need. The site contains details of the donor in a city with full address, blood group, landline and mobile telephone numbers. Anyone could save a life by enrolling himself or herself as a voluntary Blood Donor on this site. The needy could access the website and contact the donor directly during an emergency.

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