VECTOR BORNE DISEASES

EDITED BY: Dr.R.G.Wiseman Pinto
Professor & Head,
Department of Pathology,
Goa Medical College,
Ex Dean Faculty of Medicine Goa University,
President Asian Society of Cytopathology(ASC).

CURRENT TREND IN DIAGNOSIS OF VECTOR BORNE DISEASES

Dr. Savio Rodrigues

Vector borne diseases pose a significant health problem in the country with, seasonal peaks witnessed every year in various states and Goa is no exception. Apart from vectors which transmit diseases mechanically, we are more concerned about the biological vectors; where in the causative agent undergoes developmental cycle within the body of the vector.The common vector borne diseases include malaria, dengue, chikungunya, Japanese encephalitis and filariasis which accounts for considerable mortality, morbidity or both. Early diagnosis and prompt treatment is the key to tackle these problems(Apart from vector control measures) and restricting the outbreaks, as vaccination has limited scope and vaccines are not routinely used and not readily available for all vector borne diseases. More over immunity is complex and does not provide full protection in many cases; for example immunity in malaria is typically premunition immunity and last as long as infection last. The immunity is also stage specific, corresponding to various parasitic stages in the life cycle of malarial parasite and individuals living in endemic areas only are protected to some extent so long as they reside in the endemic area. Once they move out of the endemic area, they are no more protected. The most widely used vaccine against malaria is the RTS, S/ASO1 (A recombinant vaccine using Circumsporozoite protein and surface antigen of Hepatitis B), which is recommended by WHO for people living in high transmission areas like Ghana and Sub-Saharan Africa. Keeping this in mind current trends in diagnosis of common vector borne diseases are discussed

Malaria

The conventional diagnostic tool for malaria which is caused by, Plasmodium vivax, Plasmodium falciparum, Plasmodium ovale, Plasmodium malariae and rarely by Plasmodium knowlesi, is the peripheral blood smear examination, using one of the Romanowsky stain (Leishman,Wright, Giemsa , JSB or Fields). Although it is an age old technique, it is still quite effective and useful in rural settings where newer techniques are not available. Thick smears can be done after dehemoglobinization of blood sample for quick detection of parasites and thin smears can be done for ease of species identification and parasite count. In a peripheral smear the parasite is identified based on the typical

morphology and the changes that it induces in the infected red blood cells. The two common species predominantly responsible for outbreaks are Plasmodium vivax and Plasmodium falciparum and they can easily be identified based on the ring forms, with single ring form seen in infected RBCs in case of vivax and multiple rings may be seen in falciparum. In viax the infected RBCs are enlarged and show Shuffner's dot, whereas in falciparum the RBCs are normal in size and shuffner's dots are not see. There are other differentiating points also but these are good enough and more practical.

The QBC(QBC –Quantitative buffy coat), is a relatively a newer fluorescent microscopy technique which uses a florescent dye(Acridine orange) and is becoming increasingly popular as an alternative to the conventional microscopy technique.This technique is more sensitive than routine microcopy. The parasitic forms can be easily detected after staining, however, it is more expensive and its routine use as screening test may be restricted for this reason

Rapid diagnostic tests (RDTs) to demonstrate the presence of parasite specific antigens are being conveniently used for their simplicity and ease of performance. First of these tests, the ICT and Parasite "F" were exclusively used for Plasmodium falciparum species for detection of Histidine reach protein (Hrp 2) and subsequently they were redesigned to detect antigens of other plasmodium species. The tests performance needs no technical expertise and can even be done at field level. Interpretation of tests should be done with caution, taking into account that the parasitic antigens may remain in circulation 10 to 14 days after clearance of parasitaemia. These are strip based tests and hence close monitoring of positive and negative control line should also be done before recording the results. Similar strip based tests are available commercially for detection of parasite specific LDH and Aldolase enzymes. The enzyme based tests are little superior to the antigen tests as enzymes are quickly cleared from the circulation with disappearance of parasitaemia and hence severe as good prognostic marker

The antibody based tests in Malaria have limited role, and can be employed for donor screening in endemic areas and for surveillance studies but has no application in diagnostic set up.

Molecular test

The Parasite specific DNA detection tests are very sensitive and can detect as low parasitaemia as 1-2 parasite per microliter of blood , but are yet to

be considered for routine diagnostic use on account of infrastructure requirement and cost. They are very useful when species identification is disputed and is also a good research stool

Dengue

Dengue is one of the most common arbovirus infection found in India. Four species of Dengue are recognized (Den 1, Den 2, Den 3, Den 4) and 5th species have been recently identified in Bangkok. The vector involved in transmission is mainly Aedes aegypti and Aedes albopictus in some cases. A man mosquito man cycle is responsible for maintenance of infection in the community. A Sylvatic cycle that involves monkey and mosquito acts as reservoir of infection. Before taking a step forward for diagnosis of Dengue we must understand the pathogenesis of the disease so that appropriate diagnostic test is selected based on the presentation stage of the patient. After the mosquito feeds on an infected patient, it takes about 8-10 days (referred to as extrinsic incubation period) for the development of the virus within the mosquito, before the same mosquito becomes infectious and is capable of transmitting infection to another individual.The infected mosquito remains infected for life and trans ovarian transmission can occur to the offspring. After the bite from an infected mosquito the clinical manifestation are seen after another period of 8 10 days (intrinsic incubation period).

The diagnostic tests are to be interpreted with this background knowledge. Primary infections are also to be differentiated from secondary infection, as in secondary infections there is more risk of severe manifestations, such as Dengue hemorrhagic fever or dengue shock syndrome. During the primary infection two types of antibody responses are seen, the neutralizing antibodies and non-neutralizing antibodies. The neutralizing antibodies are protective and do not show cross reaction with other serotypes, while the non-neutralizing antibodies have no protective role, but in the event of subsequent secondary infection these preexisting non neutralizing antibodies cross react with other serotypes producing a cytokine response, referred to as antibody dependent enhancement (ADE), resulting in more severe clinical manifestations.

The laboratory parameters for diagnosis of dengue involve the routine blood tests and the dengue specific tests. The clinical laboratory test will show an elevated hematocrit value because of plasma leak and a decreased platelet count. Decreased serum albumin and altered liver function test may also be

noted. Microscopic hematuria may be detected in urine. Specific test are to be selected for conclusive evidence of viral infection. The first and early evidence of viral infection is by demonstration of Nonstructural protein (NS1) . This can be done using rapid strip based tests or ELISA test. The NS1 test becomes positive from day one of fever and remains positive for next 18 days. It is also an useful test to differentiate between other arbovirus infections, as there is no cross reaction of NSI with other arborvisues like the antibody test

The antibody tests can separately detect both IgM and IgG antibodies. As in all other infections IgM antibody is the first to rise followed by IgG. IgM tests become positive from 5th day onwards and may remain positive for the next 3 months in primary infections. The IgG titers may rise after 2 weeks and thereafter last for many years. A fourfold rise in the antibody titer in the antibody titer in the convalescent sample collectedat least 10 to 15 days after the primary sample is suggestive of current infection. In Secondary infections the IgM antibody response is not prominent, but the IgG positivity is seen right from day 1 of fever and will last for years, but it may show cross reaction with other arboviruses. The IgM and IgG can be detected by rapid strip tests or ELISA test

Other more reliable tests include culture of blood samples for virus isolation in mosquito cell lines and molecular tests for demonstration of viral genome. These tests are useful for demonstration of the prevailing serotypes in a given locality and useful marker for predicting severe clinical manifestation in that locality. As per CDC guidelines, for symptomatic patients during the first 1-7 days of illness, any serum sample should be tested with a Nucleic acid amplification test (NAAT) and for IgM antibody since both tests can be performed in serum. On account of availability of simpler and less cumbersome tests, Molecular tests are not routinely used for diagnosis.

Chikungunya

In India this viral infection was first reported from Calcutta since 1963 and it is showing resurgence since December 2005. The vector responsible for transmission is Aedes aegypti mosquito and the transmission cycle is similar to dengue. An anti-body test is mostly employed for routine diagnosis. IgM antibody is the first to rise within 4 days of infection and will last for the next 3 months. IgG appears after 2 weeks and last for several years. A fourfold rise in the IgG is often used as criteria for diagnosis of

recent infection. IgM (Mac ELISA) is widely used standard test for detection of early phase antibody response. Molecular methods targeted at viral RNA are used as research tools. Hematological findings of leukopenia, thrombocytopenia, raised CRP and elevated ESR are supportive finding, but does not provide any conclusive evidence.

Japanese encephalitis

Japanese encephalitis often referred to as brain fever, is reported from India since 1955. The vector responsible for its transmission in India is mainly Culex vishnui, other species like C pseudovishnui and C tritaeniorhynchus may also contribute to transmit Japanese encephalitis. Although effective vaccine is available, it is not widely used. Natural cycle of transmission exists between Ardeid birds, mosquito and pig, the latter acting as an amplifier host. Some mosquitos may leave this cycle and bite man, leading to a dead end infection in man as no man to man transmission is known.

The diagnostic approach is based on demonstration of virus/viral antigen, Isolation of the virus and demonstration of specific antibodies in the serum. Nonspecific test may also be employed as evidence for encephalitis. Demonstration of the virus may be done using immunofluorescence technique, using specific antisera tagged to a fluorescence dye. Isolation of the virus may be attempted in the early phase, from serum, CSF or brain biopsy (postmortu) using suckling mice or mosquito cell lines. These tests are of academic and research interest but are not routinely employed for diagnosis. Demonstration of four fold rise in the anti-body titer between acute and convalescent sample is often useful. The antibody detection tests IgM and IgG (Mac ELISA) are routinely employed for diagnosis. The nonspecific tests include blood tests for anemia with moderate leukocytosis and CSF examination for elevated proteins, normal or slightly raised CSF sugars with a mononuclear cellular response.

. Filariasis

A parasitic infection mainly caused by Wuchereriabancrofti and occasionally by Brugia malayi in India and Brugia timori in Timor. This infection is transmitted to man by bite of Culex fatigans, after which the third stage larva develops in man to give rise to Microfilaria, which are seen in peripheral blood within 5 -18 months after mosquito bite

Diagnosis is primarily based on demonstration of microfilaria in peripheral blood smear or other samples like lymph, chylous urine or hydrocele. This

can be attempted using unstained film or concentration technique. The samples are preferably collected at night because of periodicity exhibited by the parasite. A DEC provocation test may be done for better result

In unstained films the parasitic may be easily identified based on their motility. This motility is retained for 24-48 hours at room temperature. The stain used for staining is Romanowsky stain. QBC technique may also be used for demonstration of microfilaria. Diagnosis of occult filariasis should be kept in mind wherein the peripheral smear is negative in endemic areas and when there is strong clinical suspicion of filarial infection, this often happens because of the blockage of the lymphatics

Adult worm may also be demonstrated in biopsy specimen or in Xray examination after it is calcified.

Demonstration of antigen or antibody may be done using the rapid strip test or DOT ELISA or conventional ELISA.

DNA based tests are not as sensitive as the antigen tests and may be used to differentiate between filarial worms of human and animal origin.

Xeno-diagnostic technique, have been described where in, sterile laboratory bred mosquitos are allowed to feed on infected persons and then these mosquitoes are subsequently dissected and studied for evidence microfilaria..

Skin allergy and presence of Eosinophilia can provide additional supportive evidence

MALARIA

CLINICAL SPECTRUM AND CURRENT TREATMENT GUIDELINES

Dr. Ramnath P. Nevrekar

The classic symptom of malaria is paroxysm-a cyclical occurrence of sudden coldness

The classic symptom of malaria is paroxysm-a cyclical occurrence of sudden coldness followed by rigor and then fever and sweating, occurring every two days in P. vivax and P. ovale infections, and every three days (tertian fever) for P. malariae. P. falciparum infection can cause recurrent fever every 36–48 hours (quartan fever) or a less pronounced and almost continuous fever.

- The signs and symptoms of malaria typically begin 8–25 days following infection; signs include
- Decreased consciousness
- Significant weakness such that the person is unable to walk
- Two or more convulsions
- Low blood pressure (less than 70 mmHg in adults or 50 mmHg in children)
- Dyspnea (Pulmonary edema)
- Circulatory shock
- Kidney failure or haemoglobin in the urine
- haemoglobin less than 5 g/dl
- Low blood glucose (less than 2.2 mmol/l / 40 mg/dl)
- Acidosis or lactate levels of greater than 5 mmol/l
- A parasite level in the blood of greater than 2% □ Retinal damage, and convulsions. splenomegaly (enlarged spleen), fever without localizing signs, thrombocytopenia, and hyperbilirubinemia combined with a normal peripheral blood leukocyte count.

Rapid Diagnostic Test If a microscopy result can be made available to the provider managing the patient within same day, then only microscopy is done. Antimalarial treatment is given on the basis of a positive slide result. If a microscopy result cannot be available within same day, RDTs are to be used. RDTs are to be supplied and used for diagnosis in villages (or subcentre areas, where village data is not available) where a. Pf % > 30 and SfR > 2%: b. Consistently high API and deaths are reported. Inaccessible areas - cut off during transmission season d. Limited road and public transportation facility for treatment of severe & complicated malaria requiring immediate medical attention An RDT is done in front of the patient and a slide is taken. If the RDT

is negative, the slide is sent for microscopy. If it is positive, the patient is treated according to diagnosis and the slide is discarded in order to reduce the load on microscopy services. Wherever a microscopy result can be made available within same day, microscopy should be maintained as the only routine method. RDTs should be used in PHC and other health facilities only in emergencies in the absence of the laboratory technician (LT). It should be noted that these tests have a shelf-life of only 12 months and that they may deteriorate at high ambient temperatures.

Interpretation of Rapid diagnostic tests If a suspected malaria patient has a negative RDT, it can be assumed that the patient does not have malaria and another cause of the symptoms should be sought. If no other cause can be found and the clinical suspicion is high (e.g. intermittent fever with rigors and sweats), the test should be repeated after about 24 hours and special efforts should be made to obtain the microscopy result rapidly. 13 All fever cases diagnosed positive by either RDT or microscopy need to be promptly started on effective treatment.

The treatment will depend upon the species of Plasmodium diagnosed. The aims of the Malaria case management are:

- To provide prompt and complete treatment to all suspected/ confirmed cases of malaria

- To prevent progression of mild cases of malaria to severe or complicated form of malaria

- To prevent deaths from severe and complicated malaria

- To prevent transmission of malaria

- To minimize risk of spread of drug resistant parasites by use of effective drugs in appropriate dosage by everyone. Diagnosis and Treatment for Malaria All fever cases diagnosed as malaria by either RDT or microscopy should be promptly given effective treatment. The medicine chosen will depend upon whether the patient has vivax malaria or falciparum malaria as diagnosed by the blood test. The flow charts in different settings for diagnosis and drug selection for the treatment of malaria are as under:

Severe and complicated. Serious complications can arise in P.falciparum infection and rarely in P. vivax. They may sometimes develop suddenly over a span of time as short as 12 -24 hours and may lead to death, if not treated promptly and adequately. Severe malaria is clinically characterized by confusion or drowsiness with extreme weakness (prostration). In addition, the following may develop:

- Cerebral malaria with generalized convulsions
- Pulmonary oedema
- Severe anaemia
- Renal failure
- Hypoglycaemia
- Metabolic acidosis
- Circulatory collapse/shock
- Spontaneous bleeding and laboratory evidence of DIC
- Macroscopic haemoglobinuria
- Hyperthermia
- Hyperparasitaemia

In children, febrile convulsions, repeated vomiting and dehydration are common if the temperature is high due to any cause. Therefore, these symptoms are not necessarily indicative of severe malaria. However, children with such symptoms should be managed as severe malaria in routine program situations, and a diagnosis of malaria should be confirmed at the earliest. In pregnancy, malaria, especially P.falciparum is a serious disease because with each bout of malaria, there is a reduction in haemoglobin and profound anaemia may develop rapidly. They are also at high risk of abortions or intrauterine growth retardation because sequestration of parasites in placenta restricts oxygen and flow of nutrients to the fetus.

Drug schedule for treatment of P vivax malaria:

1. Chloroquine: 25 mg/kg body weight divided over three days i.e. 10 mg/kg on day 1, 10 mg/kg on day 2 and 5 mg/kg on day 3. Suspected malaria case Do RDT & Prepare slide Positive for P. vivax Discard slide Treat with: CQ 3 days + PQ 14 days Positive for P. falciparum Discard slide In NorthEastern states: Age-specific ACT-AL for 3 days + PQ Single dose on second day In other states: Treat with: ACT-SP for 3 days + PQ Single dose on second day Positive for Mixed infection Discard slide In North-eastern states: Treat with: Age-specific ACTAL for 3 days + PQ 0.25 mg per kg body weight daily for 14 days. In other states: SP-ACT 3 days + PQ 0.25 mg per kg body weight daily for 14 days. Negative No anti-malarial treatment However, if malaria suspected, send slide for microscopy 16 2. Primaquine*: 0.25 mg/kg body weight daily for 14 days. Primaquine is contraindicated in infants, pregnant women and individuals with G6PD deficiency. Primaquine causes haemolysis in G6PD deficient persons, resulting in dark coloured urine, yellow conjunctiva, bluish discoloration of lips, abdominal pain, nausea and vomiting, and should be reported to the doctor immediately. 14-day regimen of Primaquine should be given under supervision

Treatment of Falciparum Malaria

Artemisinin based Combination Therapy (ACT-SP)* Artesunate (AS), available as 50 mg tablets are given for three days, and SulfadoxinePyrimethamine (S-P) tablets, containing 500 mg Sulfadoxine and 25 mg pyrimethamine are given for one day, as shown in the dosage chart below. All tablets for a day should be taken together, swallowed with water. In addition, Primaquine (PQ Large) tablets should be given on the second day. Dose schedule for Treatment of uncomplicated P.falciparum cases: a. Artemisinin based Combination Therapy (ACT-SP)* Artesunate 4 mg/kg body weight daily for 3 days Plus Sulfadoxine (25 mg/kg body weight) – Pyrimethamine (1.25 mg/kg body weight) on first day. * ACT is not to be given in 1st trimester of pregnancy. b. Primaquine*: 0.75 mg/kg body weight on day 2. 18 With the introduction of different coloured Blister Packs for different age groups, treatment by the field level staff has been made easy.

Treatment of mixed infections (P.vivax + P.falciparum) cases

All mixed infections should be treated with full course of ACT and Primaquine 0.25 mg per kg body weight daily for 14 days

SP-ACT 3 days + Primaquine 0.25 mg per kg body wt. daily for 14 days

DENGUE

Mild Dengue Infection. Mild dengue infections are characterized by undifferentiated DF, fever without complications like bleeding, hypotension, organ involvement or any evidence of capillary leakage. These patients usually do not have warning signs and symptoms; hence, can be managed at home with proper counselling.

Moderate Dengue Infection Moderate dengue infections were characterized into two groups: dengue infection with warning signs and symptoms, DHF-I and II dengue infection with high-risk and comorbid conditions. Moderate dengue with warning signs and symptoms The presence of warning signs and symptoms are considered to be the indicators of severity.9 With the following warning signs and symptoms, dengue infected patients may progress into **severe stage**, which may require close monitoring.

DF with warning signs and symptoms

- Recurrent vomiting
- Abdominal pain/tenderness
- General weakness/lethargy/restlessness
- Minor bleeding
- Pleural effusion/ascites
- Hepatomegaly
- Increased hematocrit (Hct).

DHF Grade I and Grade II with or without minor bleeding: These patients are DHF without hypotension or shock. Sometimes, these patients with minor bleeding may progress to severe plasma leakage and could lead to organ involvement, massive bleeding and shock. Moderate dengue with high-risk and comorbid conditions Dengue infected patients are likely to progress to severe manifestations in presence of high-risk and comorbid conditions. The following high-risk and comorbid conditions are found to be associated with high chances of progressing to severe dengue infection: Infants Old age Diabetes Hypertension Pregnancy Coronary artery disease (CAD) Hemoglobinopathies Immunocompromised patient Patient on steroids,

anticoagulants or immunosuppressants. The moderate dengue patients should be closely monitored or possibly hospitalized for further management as these groups of patients can develop severe dengue manifestation due to abnormalities in metabolic conditions, severe plasma leakage and increases the mortality.

Severe Dengue Infection Severe dengue patients are recognized by the presence of shock, capillary leakage, significant bleeding, severe organ involvement and severe metabolic abnormalities.10 This group of patients should be immediately admitted and require intensive care management. They should be properly investigated to look for abnormalities in coagulation profile, complete hemogram and organ function test, which may require timely intravenous (IV) fluid, blood or platelet transfusion.11 Severe shock patients should be managed with fluids very carefully to prevent organ damage and pulmonary edema, which is associated with high mortality. Management of organ failure like liver, respiratory, cardiac and renal should be targeted as early as possible to prevent progression of the disease severity.12 Usually, organ failure management is done in tertiary level hospitals. Therefore, these patients should be transferred to tertiary level hospitals when indicated, without delay.

CLINICAL MANAGEMENT Approach to clinical management of DF may vary from mild, moderate and aggressive depending on severity of illness. Patients who have simple fever without any danger signs or complications may be managed with simple approach. Those who have danger signs should be managed with close monitoring for progression of DHF/DSS or severe bleeding. The patients presenting with Grade III and IV of DHF, significant bleeding or involvement of various organs will require aggressive management to reduce morbidity and mortality. Patient may develop more complications during later stage of fever (defervescence) or afebrile phase, where clinician should be careful to look for danger signs or severity of disease.

MANAGEMENT OF DENGUE FEVER Management of DF is symptomatic and supportive. Antipyretics may be used to lower the body temperature. Aspirin/NSAIDs (nonsteroidal anti-inflammatory drugs) like ibuprofen, etc. should be avoided since it may cause gastritis, vomiting, acidosis, platelet dysfunction and severe bleeding complication. Oral fluid and electrolyte therapy is recommended for patients with excessive sweating, vomiting or hypotension. Patients should be monitored for 24-48 hours in DHF endemic

areas until they become afebrile without the use of antipyretics and after hematocrit determinations are stable, platelet count is >50,000/mm3 or improving

Management of DHF (Febrile Phase) Management of febrile phase is similar to that of DF. Paracetamol is recommended to keep the temperature below 39oC. Copious amounts of fluids should be given orally, to the extent the patient tolerates, oral rehydration solution (ORS), such as those used for the treatment of diarrheal diseases and/or fruit juices are preferable to plain water. IV fluids should be administered if the patient is vomiting persistently or refusing to feed. Patients should be closely monitored for the initial signs of shock. The critical period is during the transition from the febrile to the afebrile stage and usually occurs after the third day of illness. Serial hematocrit determinations are essential guide for treatment, since they reflect the degree of plasma leakage and need for IV administration of fluids. Hematocrit should be determined daily from the third day until the temperature has remained normal for 1-2 days. Management of DHF Grade I and II Any person who has DF with thrombocytopenia and hemoconcentration and presents with abdominal pain, black tarry stools, epistaxis, bleeding from the gums, etc. needs to be hospitalized. All these patients should be observed for signs of shock. The critical period for development of shock is during transition from febrile to afebrile phase of illness, which usually occurs after third day of illness. A rise of hemoconcentration indicates need for IV fluid therapy. If patient develops fall in blood pressure (BP), decrease in urine output or other features of shock despite treatment, management for Grade III/IV DHF/DSS should be instituted. Oral rehydration should be given along with antipyretics like paracetamol, sponging, etc. as described above. The algorithm for fluid replacement therapy in case of DHF Grade I and II is given in Figure 2.Management of Shock (DHF Grade III/IV) Immediately after hospitalization, the platelet count and vital signs should be examined to assess the patient's condition and IV fluid therapy should be started. The patient requires regular and continuous monitoring. If the patient has already received about 1,000 mL of IV fluids, it should be changed to colloidal solution preferably Dextran 40/haemaccel; if the hematocrit is falling, fresh whole blood transfusion 10-20 mL/kg/dose should be given.

However, in case of persistent shock when, after initial fluid replacement and resuscitation with plasma or plasma expanders, the hematocrit continues to decline, internal bleeding should be suspected. It may be difficult to recognize

and estimate the degree of internal blood loss in the presence of hemoconcentration. Hence, whole blood in small volumes of 10 mL/kg/hour for all patients in shock as a routine precaution is recommended. Oxygen should be given to all patients in shock. Treatment algorithms for patients with DHF Grades III and IV are given in Figures 3 and 4.Calculation of Fluid The required amount of fluid should be calculated on the basis of body weight and charted on 1-3 hourly basis or even more frequently in the case of shock. For obese and overweight patients, fluid should be calculated on the basis of ideal body weight. The regimen of the flow of fluid and the time of infusion are dependent on the severity of DF. It is calculated for dehydration of about 5% deficit (plus maintenance). The maintenance fluid should be calculated using the Holiday-Segar formula

For a child weighing 40 kg, the maintenance is: 1,500 + (20 × 20) = 1,900 mL. Amount of fluid to be given in 24 hours

is calculated by adding maintenance + 5% dehydration, which is equivalent to 50 mL/kg. This should be given in 24 hours to maintain just adequate intravascular volume and circulation

Management of Severe Bleeding

In case of severe bleeding, patient should be admitted in the hospital and investigated to look for the cause and site of bleeding and immediate attempt should be made to stop the bleeding. Internal bleeding like gastrointestinal (GI) bleeding may be sometime severe and difficult to locate. Patients may also have severe epistaxis and hemoptysis and may present with profound shock. Urgent blood transfusion is life-saving in this condition. However, if blood is not available shock may be managed with proper IV fluid or plasma expander (i.e. haemaccel). If the patient has thrombocytopenia with active bleeding, it may be corrected with platelet transfusion. In case of massive hemorrhage, blood should be tested to rule out coagulopathy by testing for prothrombin time (PT) and activated partial thromboplastin time (aPTT). Patients of severe bleeding may have liver dysfunction and, in this case, liver function tests (LFTs) should also be performed. Rarely, intracranial bleed may also occur in some patients, who have severe thrombocytopenia and abnormal coagulation profile

CHIKUNGUNYA

Clinical Features: Chikungunya disease is characterized by acute transient febrile arthralgic illness, but can also lead to chronic incapacitating arthralgia. The mosquito picks up the virus from an infected person (viremia for 5 to 7 days after onset of clinical signs) during its blood-sucking meal. The virus replicates in the mosquito for a few days (extrinsic phase),and then the mosquito can transmit the virus to another person, with a new bite. Mother-to-child transmission is possible during childbirth, but not when nursing. The incubation period (time from infection to illness) can be 2-12 days, but is usually 3-7 days.46 Acute Chikungunya fever typically lasts a few days to a couple of weeks, but some patients have prolonged fatigue lasting several weeks. Additionally, some patients have reported incapacitating joint pain, or arthritis which may last for weeks or months. The prolonged joint pain associated with CHIKV is not typical of dengue. Co-circulation of dengue fever in many areas may mean that Chikungunya fever cases are sometimes clinically misdiagnosed as dengue infections, therefore the incidence of Chikungunya fever could be much higher than what has been previously reported.47 No deaths, neuro-invasive cases, or haemorrhagic cases related to CHIKV infection have been conclusively documented in the scientific literature.CHIK virus infection is most often symptomatic (H" 80%ofcases); the symptomatology may last from a few days to several years. Experts have defined 3 clinical stages: acute stage (D1 to D21), Sub-acute stage (from D21 to the end of the 3rd month), and chronic stage (after 3 months)3; The sub-acute stage and a subsequent chronic stage is not observed in all patients. Acquired immunity is usually permanent. The mortality rate of CHIK is comparable to that of seasonal influenza (H" 0. 01 to 0. 1%), and is mainly related to the patient's age (over 75 years of age) and/or to co morbidities or serious co infection. Acute stage (the first 3 weeks):A suspected case of Chikungunya is defined as a fever of sudden onset, higher than 38. 5°C, and intense arthralgia or arthritis not explained by other conditions in a person resident of or having visited an endemic or epidemic area up to two weeks before the beginning of the symptoms. In the common presentation, pyrexia occurs suddenly, along with inflammatory arthralgia and arthritis with sometimes severe pain, most frequently in the extremities (wrists, ankles, and phalanges); these symptoms last 2 to 3 weeks in some patients. Other symptoms may occur: myalgia, headache, back pain, macular to maculopapular rash, sometimes with cutaneous pruritus (foot arch) and edema of the face and extremities, polyadenopathies.48,49 Benign bleeding(gingival bleeding, epistaxis) may occur in children, but is rare in adults. Asthenia, sometimes severe, and anorexia are

common after regression of acute symptoms.FeverThe fever varies from low grade to high grade, lasting usuallyfor 24 to 48 hours. Fever rises abruptly in some, reaching39-400C, with chills and rigor, no diurnal variation, usually subsides with use of antipyretics.48Joint manifestation the joint symptoms usually start with arthralgia or arthritis. Involvement is symmetric and often ankles wrists and small joints of the hand are the worst affected. Migratory polyarthritis with effusions may be seen, but resolves in the majority. Larger joints like knee and shoulder and spine were also involved. Pain tends to be worse in the morning, relieved by mild exercise and exacerbated by aggressive movements. The pain may be relieved for 2-3 days and then reappear in a saddle back pattern. There is a tendency for early and more significant involvement of joints with some

trauma or degeneration. The classical bending phenomenon was probably due to the lower limb and back involvement which forced the patient to stoop down and bend forward

The chronic stage (after the 3rd month):It is defined by the absence of return to the pre-existing condition more than 3 months after the onset of CHIK. The chronic phase can last a few months to several years. The disease progresses to cure without sequelae, spontaneously or after treatment, or to a prolonged persistence of joint and/or general symptoms, or to aggravation because an inflammatory or degenerative process. The clinical symptomatologys are the same as in the subacute stage. It is common to observe painful rebounds on joints too strongly used considering their post-CHI inflammatory condition. The diagnostic approach is to individualized each patient according to the presence or absence of inflammatory symptoms (arthritis, enthesitis, tenosynovitis,inflammatory arthralgia) and the number of joints involved(polyarticular if ≥ 4 joints). The level of clinical inflammatory activity and its functional impact should also be taken into account.

Treatment: Guiding principles of clinical management There is no specific antiviral drug against CHIK virus. There is no specific vaccine against CHIK virus. Treatment is entirely symptomatic Paracetamol is the drug of choice with use of other analgesics if paracetamol does not provide relief. During the acute stage of the disease, steroids are not usually indicated because of the adverse effects. Aspirin is preferably avoided for fear of gastrointestinal and other side effects like Reye's syndrome. Mild forms of exercise and physiotherapy are recommended in recovering persons. Treatment should be instituted in all

suspect cases without waiting for serological or viral confirmation. It is recommended to prevent dehydration in every case (oral or parenteral fluid intake, stopping diuretics, etc. Acute phase: Acetaminophen and Other analgesic: The analgesic treatments based on acetaminophen (stage 1)in first intention. Paracetamol can be prescribed at 500-750mg, 6 hourlies, but the total daily dose should not exceed at 4g because of possible hepatotoxicity. Using NSAIDs and salicylates is not recommended in the 14 days after onset of the disease because of the risk of bleeding complications related to dengue fever unless this diagnosis is ruled out, and Reye's syndrome induced by aspirin. Using stage 2analgesics (weak opioids) is required if acetaminophen is not effective: Tramadol is a good choice when suspicious of neuropathic component of intense pain as, besides its action on opioid receptors, it acts as an antagonist of NMDA (N-methyl-D-aspartate) receptor that are involved in chronicpain.3,53 Tramadol should be used in a dose of 50-100mg, 6hourly. Codeine is an opioid that should be prescribed in a dose of 30mg every 6 hours and can be used with other analgesics. Tramadol alone or in combination with acetaminophen (adult formulation, paediatric formulation after 3 years of age); codeine combined to acetaminophen(adult fixed-dose combination (excluding breastfeeding),adding codeine to acetaminophen for children only after 12years of age (at the lowest dose and for the shortest duration);clinical safety should be monitored. Opioids: the opioid drugs are potent and safe analgesics, especially in cases of acute pain. Adverse effect monitoring is required, and the patient should be warned about adverse Effects.

VECTOR BORNE DISEASE

DR.SIVASHANKAR K.

- Malaria
- Dengue
- Chikungunya
- Filaria
- Leishmaniasis

MALARIA

- P. Vivax
- P. Falciparum
- P. Ovale
- P. Malariae
- P. Knowlesi
- Endemic in tropical zone
- Transmitting agent- female Anopheles
- Infective forms- sporozoites
- Portal of entry- skin
- Site of localization- first liver cells , then RBCs.

Life cycle

- Man – Intermediate host
- Female Anopheline mosquito- Definitive host

Phases of development in humans

- In liver

  Pre-erythrocytic schizogony

  Hypnozoite stage

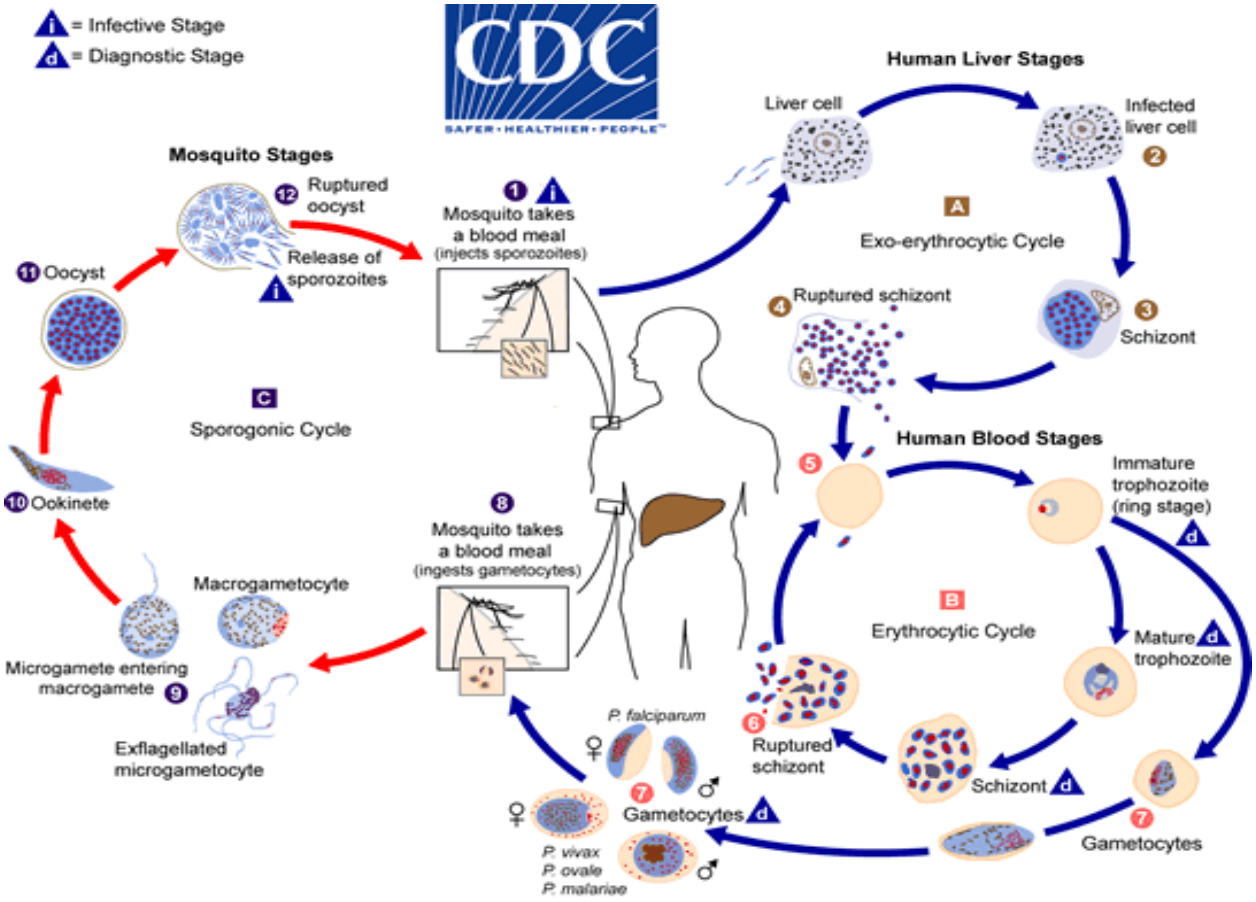
- In RBCs

  Erythrocytic schizogony

Gametogony

Development in mosquito

- Sporogony(sexual stage)
- Sexual phase (sporogony) in *Anopheles* mosquitoes that produces infectious sporozoites
- Asexual stage (schizogony) in humans that produces schizonts and merozoites
- Time required for development in the mosquito ranges from **8 - 21** days



- *P. falciparum* infects erythrocytes of all ages;
- *P. vivax* and *P. ovale* parasites primarily infect young erythrocytes;
- *P. malariae* infects older erythrocytes

Clinical features

- Defining clinical features of a malarial attack or paroxysm consist of, shaking chills, fever (up to 40° C or higher) and generalized diaphoresis, followed by resolution of fever
- Anaemia
- Splenomegaly

Malarial pathology

- Pigmentation of various organs
- Hyperplasia of recticulo-endothelial system
- Parasties filling up the capillaries
- Congestion and dilatation of sinusoidal vessels
- Degenerative changes of parenchymal cells

Pernicious malaria

- Severe complications of falciparum malaria
- Pathogenesis: capillary blockage of blood vessels

recession of asexual parasites from peripheral blood to capillary vessels of internal organs for later stages of schizogony

stickiness of infected erythrocytes to vascular endothelium.

- Types

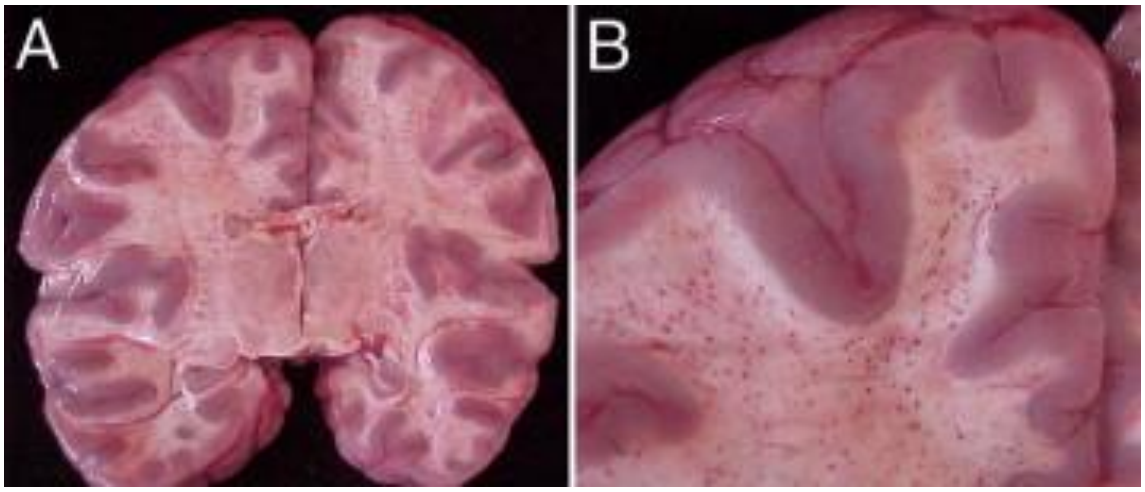
Cerebral malaria

Algid malaria

Septicaemic malaria

Cerebral malaria

- Macroscopic appearance: Multiple punctiform haemorrhages in subcortical white matter



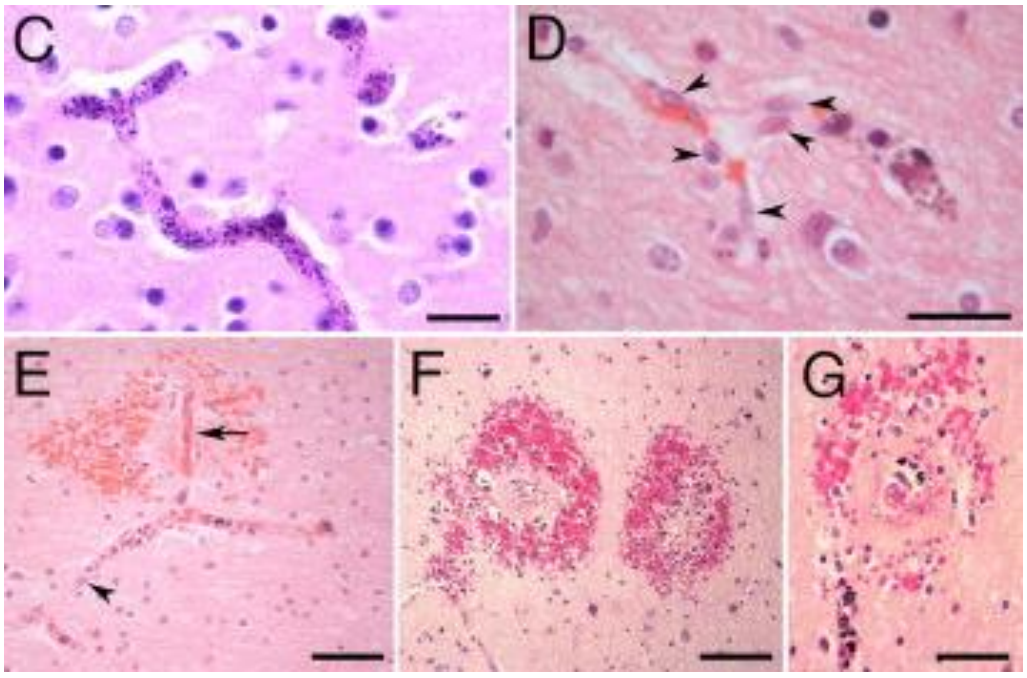
- Histology:

  dilatation and congestion of cerebral capillaries

  perivascular haemorrhages(ring haemorrhages)

  degeneration of nerve tissues

  Granuloma formation(Durck granuloma of malaria)



- Algid malaria

  cold calmmy skin with vascular collapse

- Septicaemic malaria

  high continued temperature

  pneumonia

  cardiac syncope

Clinical pathology

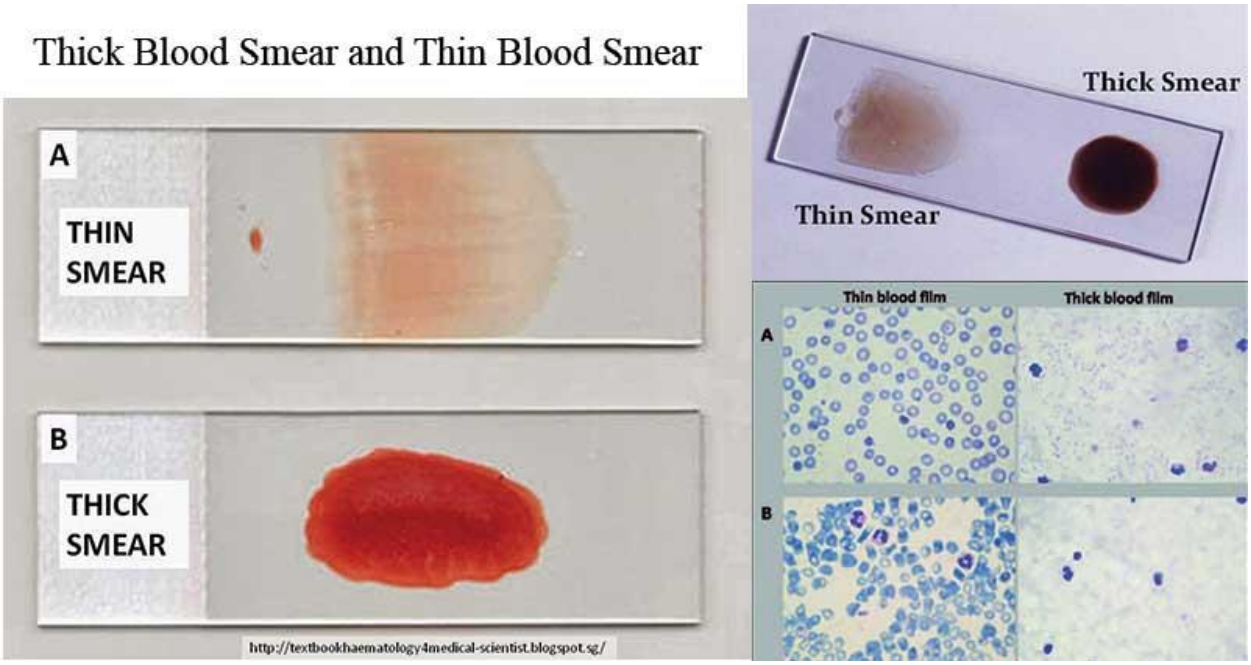
- Anaemia – destruction of infected RBCs
- Leucopenia – 3000 to 5000 per mm^3
- Platelets – decreased
- ESR is raised
- Plasma albumin is reduced.

Lab Diagnosis

- Microscopic examination
- QBC
- Rapid diagnostic test
- Molecular methods

Thick and thin smear

- Thick smear- parasite identification
- Thin smear- species differentiation



Staining

- Romanosky's stains

  Leishman's stain

  Giemsa's stain

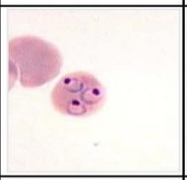
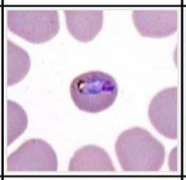
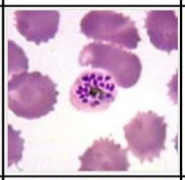
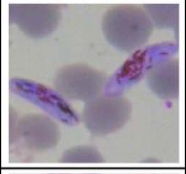
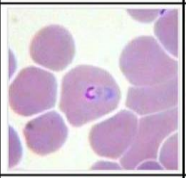
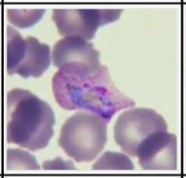
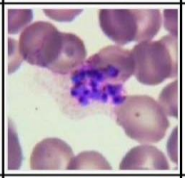
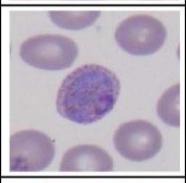
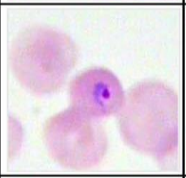
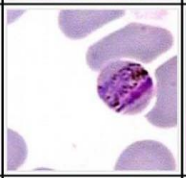
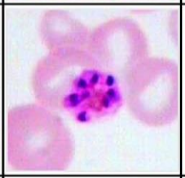
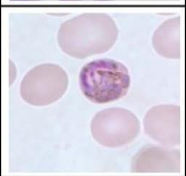
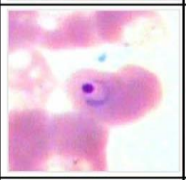
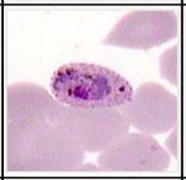
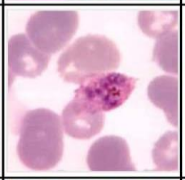
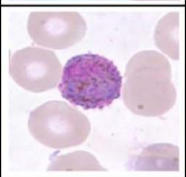
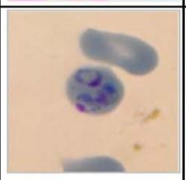
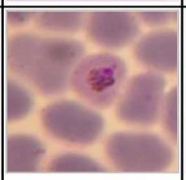
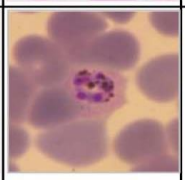
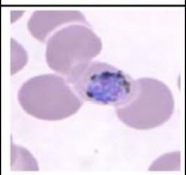
- Identification of malarial parasites on thin blood films requires a systematic approach

- 3 major factors should be considered:

  appearance of infected erythrocytes,

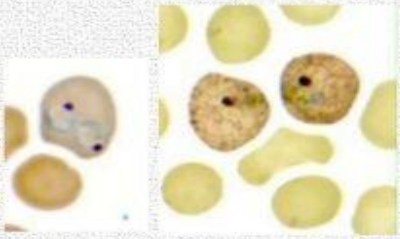
  appearance of parasites and

  stages found

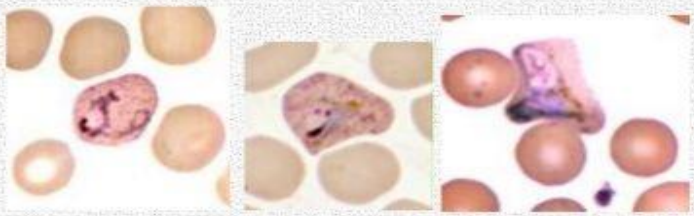
Human Malaria					
Stages / Species	Ring	Trophozoite	Schizont	Gametocyte	
P. falciparum					• *Parasitised red cells (pRBCs) not enlarged.* • *RBCs containing mature trophozoites sequestered in deep vessels.* • *Total parasite biomass = circulating parasites + sequestered parasites.*
P. vivax					• *Parasites prefer young red cells* • *pRBCs enlarged.* • *Trophozoites are amoeboid in shape.* • *All stages present in peripheral blood.*
P. malariae					• *Parasites prefer old red cells.* • *pRBCs not enlarged.* • *Trophozoites tend to have a band shape.* • *All stages present in peripheral blood*
P. ovale					• *pRBCs slightly enlarged and have an oval shape, with tufted ends.* • *All stages present in peripheral blood.*
P. knowlesi					• *pRBCs not enlarged.* • *Trophozoites, pigment spreads inside cytoplasm, like P. malariae, band form may be seen* • *Multiple invasion & high parasitaemia can be seen like P. falciparum* • *All stages present in peripheral blood.*

Plasmodium vivax

Infected erythrocytes: enlarged up to 2X; deformed; (Schüffner's dots)

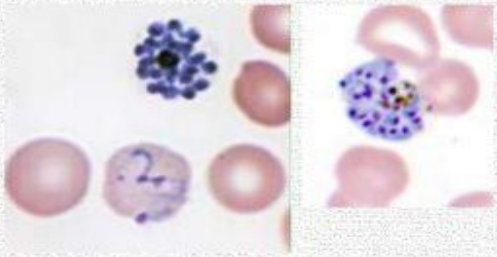


Rings



Trophozoites: ameboid; deforms the erythrocyte

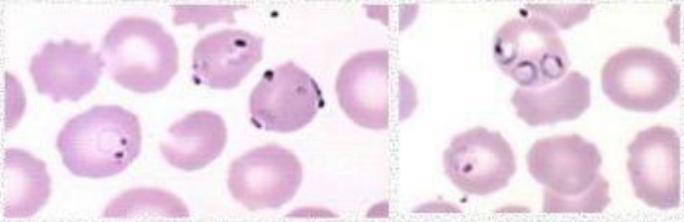
Schizonts: 12-24 merozoites



Gametocytes: round-oval

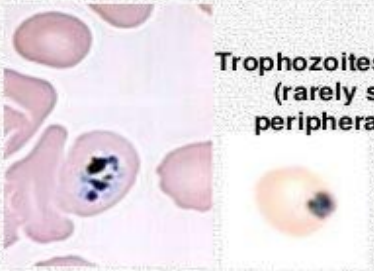
Plasmodium falciparum

Infected erythrocytes: normal size



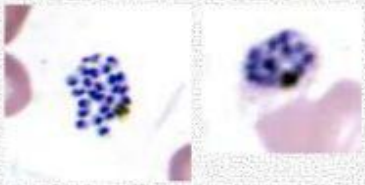
Rings: double chromatin dots; accole forms; multiple infections in same red cell

Gametocytes: mature (M)and immature (I) forms (I is rarely seen in peripheral blood)



Trophozoites: compact (rarely seen in peripheral blood)

Schizonts: 8-24 merozoites (rarely seen in peripheral blood)



Quantitative buffy coat

- The QBC test is a fluorescence-based method that concentrates parasites in a capillary tube for detection under a specialized microscope.
- Acridine orange stain

Rapid diagnostic test

- Immuno- chromatographic method
- Based on antigen detection of malarial parasite
- Histidine rich protein 2(PfHRP2)
- Parasite lactate dehydrogenase(all species)

Molecular methods

- PCR-based assays can detect and differentiate between different malaria parasite species with high sensitivity and specificity.
- These methods are particularly valuable when the parasitemia (parasite density) is low, and in cases of mixed infections.
- In June total 10 malaria case out of which 8 P. vivax and 2 P. falciparum

VECTOR BORNE DISEASES-
DENGUE,CHIKUNGUNYA AND KYASANUR FOREST DISEASE

DR. ANNA FERNANDES
DEPARTMENT OF MICROBIOLOGY

DENGUE

- Dengue virus (DENV) - **most common arbovirus** found in India.
- Belongs to family Flaviviridae.
- Enveloped virus, containing ssRNA.
- Named after the Swahili word "dinga" meaning fastidious or careful.
- Has four serotypes (DEN-1 to DEN-4) & recently, the fifth serotype (DEN-5) was discovered (2013- Bangkok)

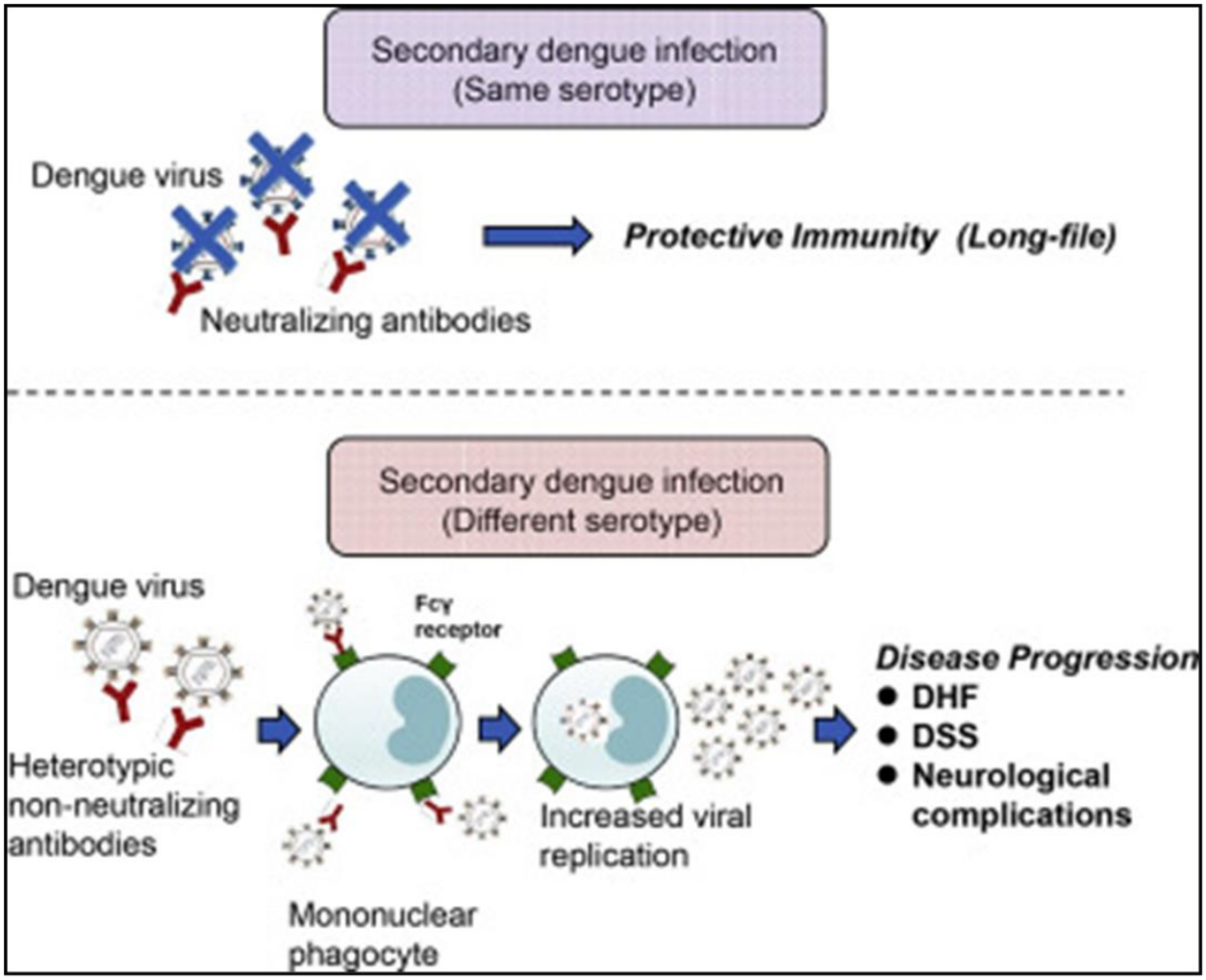
Vector

- ***Aedes aegypti*** - principal vector followed by *Aedes albopictus*.
- They bite during the day time.
- *A. aegypti* - nervous feeder (so, it bites repeatedly to more than one person to complete a blood meal) and resides in domestic places - most efficient vector.
- ***Aedes albopictus*** **-** found in peripheral urban areas - aggressive and concordant feeder; hence is less efficient in transmission.
- *Aedes* becomes infective only when it feeds on viremic patients
- Extrinsic incubation period - 8–10 days (needed before it become infective) and once infected, it remains infective for life
- *Aedes* can pass the dengue virus to its offsprings - **transovarial transmission**
- **Transmission cycle**: Man and *Aedes* - principal reservoirs.
- Transmission cycle of four serotypes do not involve other animals.
- Fifth serotype follows the sylvatic cycle.

PATHOGENESIS

- **Primary dengue infection -** infected for the first time with any one serotype.

- Months to years later, a more severe form of dengue illness may appear (called **secondary dengue infection**) due to infection with **another second serotype** which is different from the first serotype causing primary infection.



Antibody Response Against Dengue Virus

Neutralizing antibodies:

- Protective in nature.
- Such antibodies are produced against the **infective serotype** (which last **lifelong**) as well as against other serotypes (which last for some time).

Non-neutralizing antibodies

- **Last lifelong and are heterotypic** in nature - they are produced against other serotypes but not against the infective serotype.

- Such antibodies produced following the first serotype infection - bind to a second serotype during secondary dengue infection - but instead of neutralizing the second serotype, **it protects it from host immune system** by inhibiting the bystander B-cell activation against the second serotype.
- Above phenomena is called **antibody dependent enhancement (ADE)**.

Non-neutralizing antibodies (Cont..):

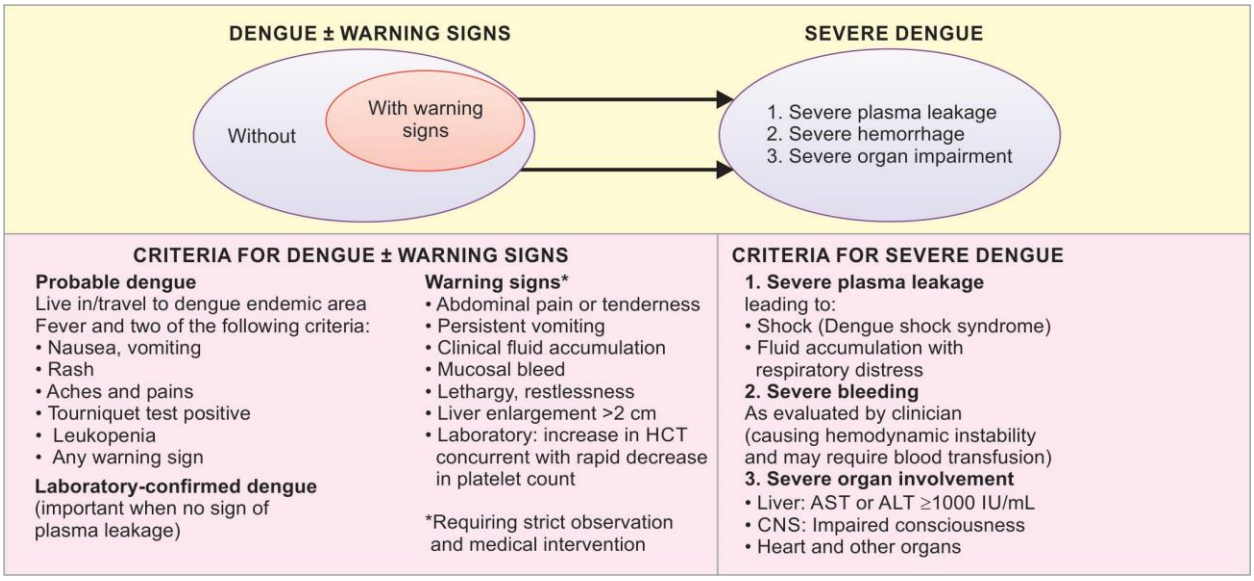
- Non-neutralizing antibodies promote recruitment of mononuclear cells followed by release of cytokines.
- Among all the serotypes combinations, ADE - remarkably observed when serotype 1 infection is followed by serotype 2, which also claims to be the most severe form of dengue infection.

Clinical Classifications

The Traditional (1997) Who Classification:

- This classification divides dengue into three clinical stages:
  - Dengue fever (DF)
  - Dengue hemorrhagic fever (DHF)
  - Dengue shock syndrome (DSS)

Dengue case classification based on the



Factors Determining the Outcome

- **Infecting serotype:** Type 2 - more dangerous than other serotypes
- **Sequence of infection:** Serotype 1 followed by serotype 2
- **Age:** Though all age groups are affected equally, children less than 12 years are more prone to develop DHF and DSS.

Dengue during Pregnancy

- Perinatal transmission of dengue infection can occur.
- Peripartum maternal infection - lead to symptomatic infection in the newborn - present with fever, thrombocytopenia, ascites or pleural effusions.
- Typically during the first week of life.

Geographical Distribution

Global Scenario:

- Endemic in more than 100 countries with 2.5 billion people at risk.
- Tropical countries of Southeast Asia and Western pacific - highest risk
- About 50 million of dengue cases occur every year worldwide, out of which 5 lakh cases (mostly children) proceed to DHF.

Situation in India:

- Prevalent in urban cities/towns affecting almost 31 states/Union territories.
- Last decade: Every year >1 Lakh cases of dengue with >200 deaths occur in India.
- In 2019: >1.37 lakh cases were reported with >130 deaths; maximum - from Karnataka and Gujarat

Situation in India (Cont..):

- All four dengue serotypes - isolated from India.
- DEN-1 and DEN-2 are widespread.

- ❖ DEN-5 has not been reported yet.

LABORATORY DIAGNOSIS

A. CLINICAL LABORATORY TESTS

- CBC - WBC Platelets haematocrit
- Albumin
- Liver function tests
- Urine - Microscopic Hematuria

B. DENGUE SPECIFIC TESTS

- Virus isolation
- Serological

Laboratory diagnosis of Dengue

NS1 Antigen Detection

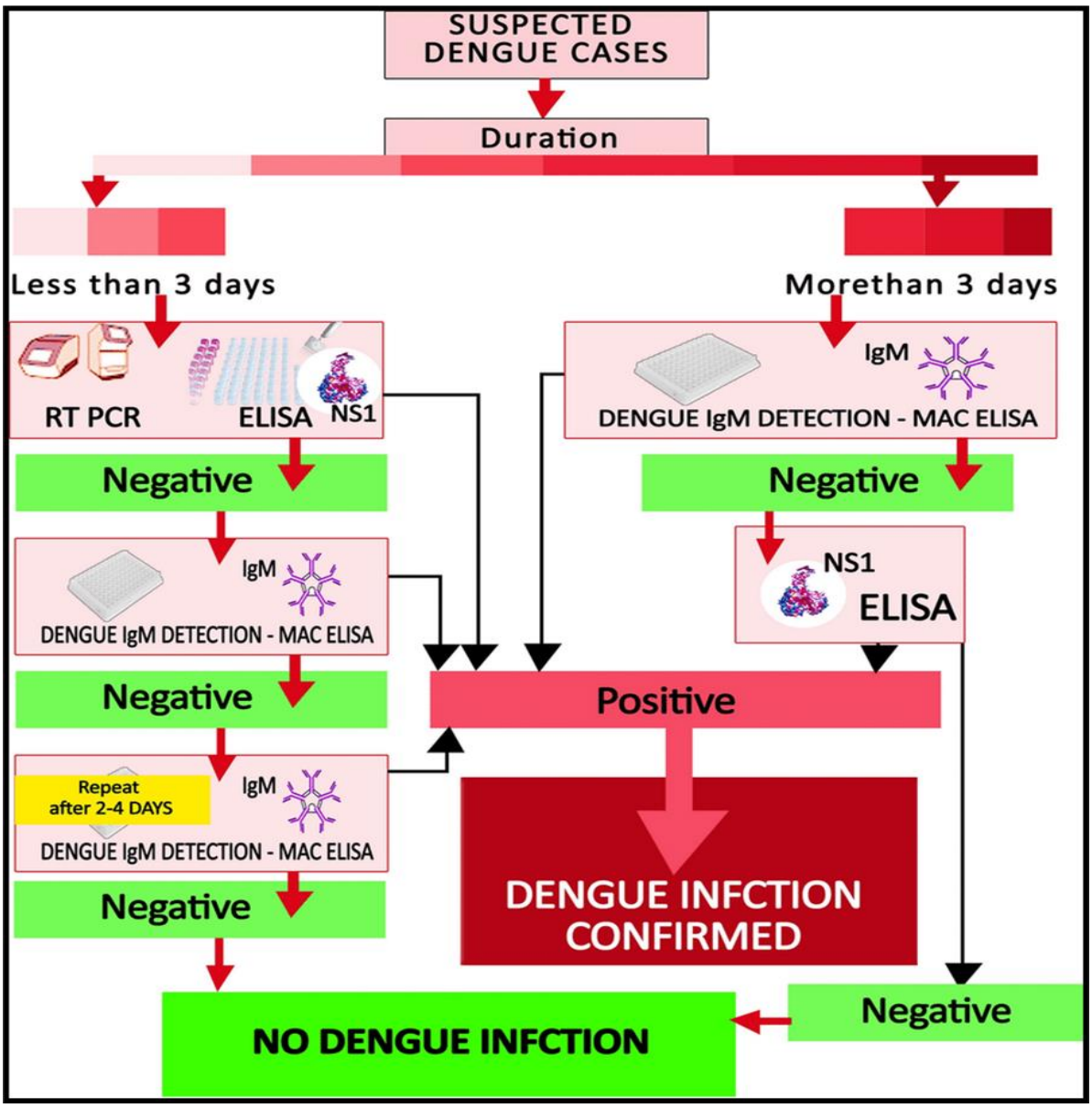
- ❖ *ELISA and ICT formats are available for detecting NS1 antigen in serum.*
- ❖ *NS1 antigen becomes detectable from day 1 of fever and remains positive up to 18 days*
- ❖ *Highly specific: It differentiates between flaviviruses. It can also be specific to different dengue serotypes.*

Antibody Detection

- ❖ *In primary infection: IgM appears first after 5 days of fever and disappears within 90 days. IgG is detectable at low titer in 14–21 days of illness, and then it slowly increases*
- ❖ *In secondary infection: IgG antibody titers rise rapidly. IgG is often cross reactive with many flaviviruses and may give false positive result after recent infection or vaccination with yellow fever virus or JE (IgM titer is significantly low and may be undetectable)*
- ❖ *In past infection: Low levels of IgG remain detectable for over 60 years and in the absence of symptoms, is a useful indicator of past infection*

Antibody Detection

- *MAC-ELISA (IgM antibody capture ELISA)*
- *Neutralization tests such as plaque reduction test, and microneutralization tests are available.*
- *RDT for dengue IgM antibodies or NS1 antigen are available - poor sensitivity and specificity. Government of India had passed an order in 2016, that a positive RDT for dengue NS1 or IgM should be considered as probable diagnosis; must be confirmed by ELISA.*
- *Virus Isolation - mosquito cell line (such as C6/36 and AP61)*
- *Molecular Method*
    - *Detection of specific genes of viral RNA (3'-UTR region) by real time RT-PCR*
    - *Genotype detection*



CHIKUNGUNYA

- ❖ Re-emerging disease characterized by acute fever with severe arthralgia.
- ❖ Belongs to family Togaviridae, of genus Alphavirus.
- ❖ Enveloped virus, containing ssRNA.

History

- The name is derived from the Makonde word “kungunyala” meaning “that which bends up or gets folded” - stooped posture which develops as a result of the severe joint pain - occurs during the course of illness

Transmission

- Human Transmission occurs by: *Aedes* mosquito, primarily *Aedes aegypti*
- Rarely - vertical transmission from mother to fetus or by blood transfusion or organ transplantation.
- Transmission cycle: Urban cycle (between human and *Aedes aegypti*) and sylvan/jungle cycle(between monkeys and forest species of *Aedes*).

Clinical Manifestations

- Incubation period is about 5 days (3–7 days).
- Acute stage:
  - Arthritis - polyarticular, migratory and edematous (joint swelling), predominantly affecting the small joints of wrists and ankles
  - Other symptoms - headache, muscle pain, tenosynovitis or morbilliform skin rashes
  - Chik sign (also called brownie nose appearance)

Manifestations of chikungunya and dengue

Features	Chikungunya	Dengue
Fever (onset, duration)	Acute, 2–4 days	Gradual, 5–7 days
Polyarthritis	Frequent, May last longer (>1 month)	Less common Short duration
Tenosynovitis	Common	None
Rashes appear on	Day 1–4, maculopapular	Day 3–7, Petechiae or maculopapular
Myalgia	Possible	Common
Leukopenia	Common	Infrequent

Features	Chikungunya	Dengue
Thrombocytopenia	Infrequent	Common
Retro-orbital pain	Rare	Common
Hypotension and shock	Possible	Common
Minor bleeding	Rare	Common
Hematocrit	Normal	Increased

Epidemiology

- First reported in Africa (Tanzania, 1952) - subsequently introduced into Asia and had caused several outbreaks in various African and Southeast Asian countries (Bangkok and India).
- India (past): Several outbreaks were reported during 1963–1973; e.g. Kolkata in 1963 and South India in 1964 (Puducherry, Chennai-Vellore region) and Barsi in Maharashtra in 1973
- India (at present): Chikungunya is endemic in several states
- States: Karnataka, Tamil Nadu, Andhra Pradesh and West Bengal.
- In 2019, nearly 65,217 suspected and 9,477 confirmed cases were reported.
- Karnataka accounted for the maximum number of cases followed by Maharashtra.

Reasons for Re-emergence

- Re-emergence in 2005 - believed to be due to a novel mutation in the virus and a change in vector.
- New mutation (E1-A226V): Alanine in the 226 position of E1 glycoprotein gene is replaced by valine.
- New vector (*Aedes albopictus*): This mutation led to a shift of vector preference.

Laboratory Diagnosis

- Viral isolation - mosquito cell lines (takes 1–2 weeks)
- Serum antibody detection – MAC ELISA
    - IgM appears after 4 days of infection and lasts for 3 months; IgG appears late (after 2 weeks) and lasts for years.
    - So, detection of IgM or a fourfold rise in IgG titer is more significant
- Molecular method
- Hematological finding - leukopenia with lymphocyte predominance, thrombocytopenia (rare), elevated ESR and C-reactive protein

KYASANUR FOREST DISEASE

- Was identified in 1957 from monkeys from the Kyasanur Forest in Shimoga district of Karnataka, India.
- It belongs to the family Flaviviridae.
- Enveloped virus, containing ssRNA.

Epidemiology

- Vector: Hard ticks (*Haemaphysalis spinigera*)
- Hosts: Monkeys, rodents and squirrels - maintain the virus through animal-tick cycles.
- Monkeys are the amplifier hosts, where the virus multiplies exponentially
- Seasonality: KFD is increasingly reported in dry months (November to June) which coincides with human activity in forest
- Situation in India: Endemic in five districts of Karnataka - Shimoga, North Kannada, South Kannada, Chikkamagaluru and Udupi

Clinical Manifestation in Humans

- Incubation period varies from 3–8 days
    - First stage - hemorrhagic fever
    - Second stage - meningoencephalitis

Laboratory Diagnosis

- Virus isolation from blood
- IgM antibody detection by ELISA.
- Nested RT-PCR and real time RT-PCR