5. LABORATORY INVESTIGATIONS

5.1 Disease Monitoring Laboratory Tests

Full Blood Count (FBC)

1. White cell count (WCC):

In the beginning of the febrile phase WCC is usually normal but will decrease rapidly as the disease progresses.[10] WCC may show relative lymphocytosis, this non-specific trend of leucopenia should raise the suspicion of possible dengue infection in appropriate settings.

2. Hematocrit (HCT):

Hemoconcentration as depicted by rising HCT is a marker of plasma leakage in dengue infection and helps to differentiate between DF and DHF –A significant blood loss and early fluid replacement may mask this trend.[36] It is of paramount importance to get a **baseline HCT in the early febrile phase of disease.** It will become handy latter on for early recognition of plasma leak – detection of rising HCT level.

3. Thrombocytopenia:

Thrombocytopenia is perhaps the most common (and most maligned) laboratory investigation in dengue infection.[36] In the early febrile phase, platelet count is usually within normal range but it will decrease rapidly as the disease progresses to the late febrile phase or at defervescence and it may continue to remain low for the first few days of recovery. There is a significant negative correlation between disease severity and platelet count [8, 45] but it is not predictive of bleeding.[46, 47, 48, 49, 50]

4. Liver Function Test

Abnormal LFTs in the form of elevated transaminases is common and is characterized by greater elevation of the AST as compared to the ALT.[51] The frequency and degree of elevation of the liver enzymes are higher with DHF compared to DF.[51, 52]

- Leucopenia followed by progressive thrombocytopenia is suggestive of dengue infection.
- A rising HCT accompanying progressive thrombocytopenia is suggestive of DHF.
- There is no local data available on the normal range of HCT in adults. In the absence of a baseline HCT level, a HCT value of >40% in female adults and >46% in male adults should raise the suspicion of plasma leakage.

Recommendations

- The baseline HCT and WCC should be established as early as possible in all patients with suspected dengue.
- Serial FBC and HCT must be monitored as the disease progresses.

5.2 Diagnostic Tests

Definitive diagnosis of dengue infection can only be made through laboratory investigations. **Interpretation of laboratory dia gnostic results, how ever, should only be done in the clinical context.**

Laboratory confirmatory tests include antibody detection (serology), virus isolation, detection of virus genetic materials (polymerase chain reaction -PCR) and detection of dengue virus protein (NS1 antigen).

5.2.1 Dengue Serology Tests

Hemagglutination Inhibition Test

The hemagglutination Inhibition (HI) test: HI test is considered a gold standard for the serological diagnosis of dengue. It is non-mechanized labor intensive test which requires paired samples for proper interpretation, therefore it is a test that is mainly being used for research - to differentiate between primary and secondary dengue infections.

Dengue IgM test: Dengue-IgM capture enzyme-linked immunosorbent assay (ELISA) is the most widely used serological test, to diagnose dengue. The titer of IgM tends to be significantly higher in primary infections (1^0) as compared to secondary (2^0) infections.[53] Once the IgM becomes detectable by day 5, it rises rapidly and peaks at

about 2 weeks after the onset of symptoms, it wanes slowly in the following months to reach undetectable levels after a variable interval. Some fully recovered healthy people, who had had an exposure to the dengue virus in the recent past, might well test positive to the dengue IgM. Therefore, mere presence of IgM might not be diagnostic of a current illness.[54] A positive IgM result, in endemic situation, therefore, has to be interpreted with care taking the clinical picture in to consideration. If the dengue IgM test is the only available diagnostic test in the hospital, **a paired sample** - one in early febrile phase and other later, after day 5 of illness - will be essential for proper interpretation of the results.[54]

Specific IgM was detected in all the cases with primary dengue virus infection on disease day 9 or later.[53] Anti-dengue IgM is, a sensitive test for detection of 1^0 infection of dengue after day seven – (IgM was detected in only 55% of patients with primary dengue infections between day 4-7 of the onset of fever) - which became positive in 100% of the patients after day seven to nine.

In 2^0 dengue infections, IgM was detected in only 78% of patients after day seven.[55] In another study, 28% of secondary dengue infections remained undiagnosed when IgM was the only test performed.[9, 56, 57] It can be assumed, therefore, that IgM is not very reliable test for detection of secondary dengue infection.

Indirect IgG ELISA test: Both in1^0 and 2^0 dengue infection, dengue IgG becomes detectable in 100% of patients after day seven of onset of fever. A paired dengue IgG is, therefore, a recommended test to see the seroconversion; if dengue IgM stays negative after day seven, if the disease is still suspected clinically.[55, 56]

Recommendations

- Seroconversion for dengue IgM in a **paired sample** is conclusive evidence of dengue fever. Therefore dengue IgM should be taken as soon as the disease is suspected.
- Dengue IgM is usually becomes positive after day 5-7 of illness. Therefore a negative IgM taken before day 5-7 of illness does not exclude dengue infection.
- If dengue IgM is negative before day seven, a repeat sample must be taken in recovery phase.
- If dengue IgM is still negative after day seven with negative IgG test reported at less than seven days, a four fold rise in reciprocal Ig G antibody titre between acute and convalescent sera is needed for diagnostic confirmation. **Appendix 4b**

Simple rapid tests such as the strip assays (immune-chromatography test) are available for qualitative detection of dengue IgM and IgG. These rapid tests have moderate sensitivity and specificity when the samples are collected in the late convalescent phase. These can be used **when ELISA test were not available**[60] But have to be interpreted within the clinical context with clear understanding of significantly reduced sensitivity and specificity.[59, 60, 57, 61] It is recommended that the dengue IgM ELISA test be done after a rapid test.[59]

Note: False positive dengue serology:

Serological tests for dengue have been shown to cross-react with:

• Other flavivirides – Japanese Encephalitis.[62, 57]

• Non-flavivirus infections– malaria, leptospirosis, toxoplasmosis, syphilis.[63, 60]

• Connective tissue diseases – rheumatoid arthritis.[59]

5.2.2 Virus Isolation

The definitive test for dengue infection is the virus isolation. However this test can only be performed in specialized labs equipped with tissue culture and virus isolation facilities. It is useful only at the early viremic phase of the illness. Generally, virus can be detected in the blood until day five of illness; i.e. before the formation of neutralizing antibodies.

During the febrile illness, dengue virus can be isolated from serum, plasma and leucocytes. It can also be isolated from post mortem specimens. The monoclonal antibody immunofluorescence test is the method of choice for identification of dengue virus. This costly test may take up to two weeks to complete.

Note: Virus isolation has a poorer yield as compared to PCR. It is most probably due to poor viability of the virus and the poor quality of the samples.[64]

5.2.3 Polymerase Chain Reaction (PCR)

In the early phase (< 5 days of illness), molecular tests such as the reverse transcriptase – polymerase chain reaction (RT- PCR) can be very useful for the diagnosis of dengue infection. It was shown to have a sensitivity of 100% in the first 5 days of disease, but is reduced to about 70% by the day six, as expected with declining

viraemia.[65, 66, 67] An additional advantage of RT-PCR is the ability to determine dengue serotypes [68, 66, 69, 64, 70]

Limitations of RT- PCR are:

a) This test is only available in a few centers with facilities and trained personnel (e.g. NIH Islamabad, AIMC, IPH and Services Institute of Medical Sciences, Lahore).

b) The test is costly.

c) The specimen requires special handling for storage and transport, between the time of collection and extraction (**Appendix 8**) In view of these limitations, the use of RT-PCR should only be considered for in-patients who present with diagnostic challenges in the early phase of illness.

5.2.4 Non-Structural Protein-1 (NS1 Antigen)

NS1 antigen is a highly conserved glycoprotein that seems to be essential for virus viability. Secretion of the NS1 protein is a hallmark of flavivirus infecting mammalian cells and **can be found in dengue infection as well as in yellow fever and West Nile virus infection**. This antigen is present in high concentrations in the sera of dengue infected patients during the early phase of the disease.[71, 72] The detection rate is much better in acute sera of primary infection (75%- 97.3%) when compared to the acute sera of secondary infection (60% 70%).[73, 74, 75, 76]

The sensitivity of NS1 antigen detection starts to drop off from the day 4-5 of illness and is usually undetectable in the convalescence phase.[67, 76, 74, 75]

Recommendations

- PCR can be used as a diagnostic tool in early dengue infection. It is not recommended as a routine diagnostic test due to limited availability and cost.
- NS1 Ag is a good diagnostic tool that is very useful in the early phase of dengue infection. It is not useful in the convalescence phase.

Please refer to **Appendix 8** for methods of sample collection for diagnostic tests