Letter to editor doi:

Detection of dengue NS1 antigen, alongside IgM plus IgG and concurrent platelet enumeration during an outbreak

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During the late incubation period or initial phase of dengue virus infection, laboratory confirmation is through viral isolation in cell culture and/or molecular investigations, or immunofluorescence, or immunohistochemistry[1]. The dengue virus non-structural antigen NS1 that would develop before the appearance of dengue IgM and/or IgG is emerging as a suitable option for dengue diagnosis[2]. Platelet therapy is a standard clinical practice for dengue patients with severe thrombocytopenia[3]. However, during introductory screening, platelet count is not being done in many cases. This results in delays of platelet therapy.

In the course of the current (2010) spurt of dengue in New Delhi[4], simultaneous screening for NS1, IgM and IgG and platelet enumeration has been introduced at the Sant Parmanand Hospital, a 140-bedded tertiary care, private hospital in Delhi.

During the last fortnight of August 2010, samples from 175 suspected patients were taken for testing by the combined 'dengue package' that included assays for NS1, IgM, IgG and automated platelet enumeration. Blood samples were drawn into EDTA tubes and tested for dengue components employing the single-step method: immunochromatigrahic one step dengue NS1 Ag and IgG/IgM test, dengue duo, in accordance with the manufacturer's instructions (Standard Diagnostics, Inc, www.standardia.com). The platelet enumeration was in the 5- or 3-differential analyzers, Coulter^{R}A^{c} T^{Tm} 5diff Autoloader (Beckman Coulter, Fullerton, CA) or BC-3000Plus (Mindary, Shenzhen, China). The reports were available within 1-2 hours.

Patients' age ranged from 6 months to 73 years, mean 28.7 years: standard deviation=13.4, SEM=1.01. A total of 86 patients were NS1-positive in different combinations, while 89 were NS1-negative. Among 86 NS 1-positive patients, 29 were IgM-positives and 57 were IgG-negative. Ten were IgG-positive and 76 were IgG-negative. Four patients with IgG- positive were also IgM- positive. There were 9 IgM-positive patients and 80 IgM-negative in 89 NS1-negative patients; while 15 were IgG-positive and 74 IgG-negative. There were 6 patients who were positive to three markers while 72 were negative and 53 patients were positive exclusively to NS1.

The platelet count among 86 NS1-positives [(115±6) $\times 10^9$/L] was lower than the 89 NS1- negatives[(149±11) $\times 10^9$/L] (P=0.0086). Counts of 29 patients co-positives to NS1 and IgM was (102±10)$\times 10^9$/L, counts of 15 patients with NS-1 negative but IgG- positives was (44±9)$\times 10^9$/L , 9 patients with NS1- negative and IgM-positive was (62±16)$\times 10^9$/L; count of 6 patients with NS1 positive, IgM positive and IgG positive was (76±27) $\times 10^9$/L; count of 10 patients with NS1 positive and IgG positive was (89±21)$\times 10^9$/L.

86 NS1-positive patients included 57 who were negative for IgM and would have otherwise been missed. Four exclusive IgG-positives, with a secondary viral infection, would have been overlooked. The NS1 test is helpful in dengue diagnosis, otherwise they would be labeled as negative. The concurrent NS1-positive and IgM positive status of 29 patients reinforced the serological diagnosis during the earlier phase of illness.

It would not be possible to rule out dengue infection in above 72 triple-negative cases without testing for viral replication in cell culture and/or molecular investigations or immunofluorescence, or immunohistochemistry[1], for which facilities are not available in our hospital. Such patients should be investigated for acute febrile illnesses including malaria, urinary tract infection, enteric fever, Chikungunya virus and the influenza virus H1N1 infection.

With dengue package, a single laboratory test for serology and platelet enumeration has been an asset for clinicians and patients. Based of results, platelet transfusion can be started straightaway.

There has been a wide variation in the platelet counts of the patients. The platelet count in 8 NS1-negatives was <20$\times 10^9$/L, 4 in NS1-positives, 1 in patients with NS1 and IgM positive, 3 in patients with NS1 negative but IgM positive, 6 in patients with NS1 negative but IgG positive, 1 in patients with NS1, IgM and IgG positive, and 2 in patients with NS1 and IgG positive. This would help to differentiate between patients with dengue-associated thrombocytopenia and those with severe bleeding episodes associated with trauma, invasive intensive care procedures or emergency surgery. Moreover, in patients with platelet count <20 $\times 10^9$/L, decreased megakaryocytic production, splenic sequestration, non-immune or immune destruction of platelets should be examined.

To conclude, use of dengue package is precious in dengue virus diagnosis in health care centres lacking sophisticated laboratory facilities. This would help physicians to offer rational therapy to patients, and government agencies to initiate vector control measures and active disease surveillance.

References

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