JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2010, p. 4688–4689 Vol. 48, No. 12
0095-1137/10/$12.00 doi:10.1128/JCM.01668-10
Copyright © 2010, American Society for Microbiology. All Rights Reserved.

Nonstructural Protein NS1: Giving a New Structure to Dengue Diagnosis

In a recent article (6), Wang et al. reported an extensive evaluation of NS1 antigen and compared it with real-time PCR, an IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA), and a hemagglutination inhibition assay for diagnosis of dengue virus infection. The authors reported MAC-ELISA positivity for 46 (92%) out of 50 samples positive for dengue virus by real-time PCR (RT-PCR). However, the literature suggests (5) that viremia in dengue lasts for less than 5 days and that the IgM antibody response takes 5 to 10 days to develop in cases of primary dengue virus infection and 4 to 5 days in cases of secondary dengue virus infection (2, 5). Hence, at a particular time postinfection, it is unlikely that the results for both of these tests will be positive. The authors report that they were able to detect viral RNA up to 8 days postinfection. However, this also does not explain the overlap period, as it does not explain why RT-PCR positivity was observed in only 1 out of 100 dengue virus IgM-positive samples. It is important to know in how many cases the virus genome could be detected until day 8 out of the 50 samples that were positive by real-time PCR. It would also be valuable to know whether the same patient's blood was drawn for 8 consecutive days and checked for viremia and, if so, whether the virus was detectable.

The authors mention that they were able to detect NS1 antigen up to 14 days from the onset of fever. Previous studies (1, 3) have reported that NS1 antigen is positive for 9 days postinfection in cases of primary dengue and up to 5 days postinfection in cases of secondary dengue.

We have also evaluated and compared NS1 with IgM for diagnosis of acute dengue virus infection in 87 patients. NS1 antigen had a sensitivity of 71 to 100% when used for patients who had a fever for 3 days. We suggest that NS1 antigen should be considered the test of choice for patients presenting with a history of fever of up to 3 days of duration. On day 4, a combination of MAC-ELISA and an NS1 antigen test would increase the sensitivity of diagnosis. We found that detection of NS1 antigen was not useful beyond day 4 of fever. We also compared both of these diagnostic modalities with conventional RT-PCR for 40 samples from patients with acute dengue. The results for the NS1 test were positive for an additional 15 samples which were negative by conventional RT-PCR (4). It is important to know that NS1 testing is also available as a strip test and also as a dengue duo test (NS1/IgM/IgG), which has further increased the sensitivity of detection.

REFERENCES

1. **Alcon, S., A. Talarmin, M. Debruyne, A. Falconar, V. Deubel, and M. Flamand.** 2002. Enzyme-linked immunosorbent assay specific to dengue virus type 1 nonstructural protein NS1 reveals circulation of the antigen in the blood during the acute phase of disease in patients experiencing primary or secondary infections. J. Clin. Microbiol. **40:**376–381.
2. **Gubler, D. J., W. Suharyono, R. Tan, M. Abidin, and A. Sie.** 1981. Viraemia in patients with naturally acquired dengue infection. Bull. World Health Organ. **59:**623–630.
3. **Kumarasamy, V., A. H. Wahab, S. K. Chua, Z. Hassan, Y. K. Chem., M. Mohamad, and K. B. Chua.** 2007. Evaluation of a commercial dengue NS1 antigen-capture ELISA for laboratory diagnosis of acute dengue virus infection. J. Virol. Methods **140:**75–79.
4. **Singh, M. P., M. Majumdar, G. Singh, K. Goyal, K. Preet, A. Sarwal, B. Mishra, and R. K. Ratho.** 2010. NS 1 antigen as an early diagnostic marker in dengue: report from India. Diagn. Microbiol. Infect. Dis. **68:**50–54.
5. **Vaughn, D. W., S. Green, S. Kalayanarooj, B. L. Innis, S. Nimmannitya, S. Suntayakorn, A. L. Rothman, F. A. Ennis, and A. Nisalak.** 1997. Dengue in the early febrile phase: viremia and antibody responses. J. Infect. Dis. **176:** 322–330.
6. **Wang, S. M., and S. D. Sekaran.** 2010. Evaluation of a commercial SD dengue virus NS1 antigen capture enzyme-linked immunosorbent assay kit for early diagnosis of dengue virus infection. J. Clin. Microbiol. **48:**2793–2797.

Mini P. Singh*
Department of Virology
Postgraduate Institute of Medical Education and Research
Chandigarh 160012, India

Kapil Goyal
Department of Microbiology
Postgraduate Institute of Medical Education and Research
Chandigarh 160012, India

R. K. Ratho
Department of Virology
Postgraduate Institute of Medical Education and Research
Chandigarh 160012, India

*Phone: 91-9357784144
Fax: 81-172-2744401
E-mail: minipsingh@gmail.com

Authors' Reply

In our study, the IgM antibody response was observed to develop on day 2 after the onset of illness. The rapidity with which IgM develops differs considerably among patients. Although most patients had detectable IgM antibody by day 5 of illness, some patients developed IgM on day 3 or 4 after the onset of illness, whereas others may develop IgM only 7 to 8 days after the onset. Most of our cases were secondary dengue cases. Also, we note that in secondary cases the overwhelming IgG response can block detection of IgM. We therefore cannot agree that at a particular time postinfection it is unlikely that both RT-PCR and MAC-ELISA will give positive results. We have also shown this in our previous publications indicating that viral RNAs were detected by a real-time assay in about 70% of our IgM-positive samples (3, 6). In our current study, 46 (92%) out of the 50 confirmed RT-PCR-positive samples tested positive by MAC-ELISA (Table 1). However, for the overall 320 study subjects, 88 (54.3%) out of the 162 RT-PCR positive samples tested positive by MAC-ELISA (Table 1).

The proper handling and storage of specimens affect the sensitivity of RT-PCR and virus isolation. In addition, the level of viremia differs greatly, depending on the time after onset, the antibody titers, and/or the strain of the infecting virus. Although viremia is usually detectable for an average of 4 to 5 days, in our study, the virus genome could be detected until day 8. As for doing daily blood collection, it is not feasible to draw blood for 8 consecutive days, as the blood collected was obtained from outpatient or hospitalized patients from our dengue surveillance study.

Previous studies by Alcon et al. (1), Dussart et al. (2), and Kumarasamy et al. (4) have reported that the NS1 antigen was detectable from the first day after the onset of the fever up to day 9. Although our study has shown that NS1 antigen was detectable 14 days after onset of the fever, this is in agreement with findings from Xu et al., whose data suggested that the NS1 antigen, limited to dengue virus serotype 1, was detectable until day 18 after the onset of the symptoms (5).

TABLE 1. Samples positive for both RT-PCR and MAC-ELISA on days 1 through 8 after the onset of illness

Day after the onset of illness	No. of confirmed RT-PCR-positive samples from the current study		No. of RT-PCR-positive samples from the overall 320 study subjects	
	Total	Positive by MAC-ELISA	Total	Positive by MAC-ELISA
1	0	0	7	0
2	2	1	17	2
3	7	7	42	22
4	16	14	47	24
5	15	14	35	28
6	4	4	7	5
7	1	1	1	1
8	5	5	6	6
Total	50	46	162	88

In our opinion, all evaluation studies should follow standard criteria, including correct sample grouping in accordance with the gold standard and a minimum number of samples in each group. It should be understood that different populations respond differently immunologically. The world is now quite heterogenous, and therefore, blanket inferences cannot apply. In our opinion, several of the studies cited include too few samples to be valid. The minimum number that we would suggest is 250 to 300 samples per evaluation. Even then, only suggestions can be given. To determine what tests are appropriate for a certain population, one must take into account the endemicity in the regions, the population at risk, their genetic background, and their living conditions. All of this is going to affect the immunological response.

REFERENCES

1. **Alcon, S., A. Talarmin, M. Debruyne, A. Falconar, V. Deubel, and M. Flamand.** 2002. Enzyme-linked immunosorbent assay specific to dengue virus type 1 nonstructural protein NS1 reveals circulation of the antigen in the blood during the acute phase of disease in patients experiencing primary or secondary infections. J. Clin. Microbiol. **40:**376–381.
2. **Dussart, P., B. Labeau, G. Lagathu, P. Louis, M. R. Nunes, S. G. Rodrigues, C. Storck-Hermann, R. Cesaire, J. Morvan, M. Flamand, and L. Baril.** 2006. Evaluation of an enzyme immunoassay for detection of dengue virus NS1 antigen in human serum. Clin. Vaccine Immunol. **13:**1185–1189.
3. **Kong, Y. Y., C. H. Thay, T. C. Tin, and S. Devi.** 2006. Rapid detection, serotyping and quantitation of dengue viruses by TaqMan real-time one-step RT-PCR. J. Virol. Methods **138:**123–130.
4. **Kumarasamy, V., A. H. Wahab, S. K. Chua, Z. Hassan, Y. K. Chem, M. Mohamad, and K. B. Chua.** 2007. Evaluation of a commercial dengue NS1 antigen46 capture ELISA for laboratory diagnosis of acute dengue virus infection. J. Virol. Methods **140:**75–79.
5. **Xu, H., B. Di, Y. X. Pan, L. W. Qiu, Y. D. Wang, W. Hao, L. J. He, K. Y. Yuen, and X. Y. Che.** 2006. Serotype-specific monoclonal antibody-based antigen capture immunoassay for detection of circulating nonstructural protein NS1: implications for early diagnosis and serotyping of dengue virus infections. J. Clin. Microbiol. **44:**2872–2878.
6. **Yong, Y. K., R. Thayan, H. T. Chong, C. T. Tan, and S. Devi.** 2007. Rapid detection and serotyping of dengue virus by multiplex RT-PCR and real-time SYBR green RT-PCR. Singapore Med. J. **48:**662–668.

Shamala Devi Sekaran*
Seok Mui Wang
Department of Medical Microbiology
Faculty of Medicine
University of Malaya
50603 Kuala Lumpur, Malaysia

*E-mail: shamalamy@yahoo.com