False-Positive Transcription-Mediated Amplification Assay Detection of West Nile Virus in Blood from a Patient with Viremia Caused by an Usutu Virus Infection

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Received 24 December 2009/Returned for modification 20 April 2010/Accepted 23 June 2010

Detection of West Nile virus (WNV) by nucleic acid amplification technology (NAAT) is used widely to screen blood and organ donations in areas where WNV is endemic. We report a false-positive result of a WNV transcription-mediated amplification assay (TMA) in a patient with viremia that was caused by Usutu virus, a mosquito-borne flavivirus.

Certain viruses are transmissible by blood transfusion. Of those that have been examined with regard to the safety of blood donations, some are new and emerging pathogens, such as West Nile virus (WNV). This virus causes severe disease in humans who receive infected blood transfusions (1). WNV is a flavivirus and is closely related to other human-pathogenic viruses, including dengue virus (DENV), yellow fever virus (YFV), and Japanese encephalitis virus (JEV) (6). Most cases of WNV infection remain asymptomatic, and a limited number of infections become clinically evident as severe neuroinvasive disease (6).

In October 2008, we reported the first human case of WNV illness to occur in Italy (11). This patient was identified during the active surveillance program of blood and organ donations that the Public Health Authority of the Emilia Romagna region initiated in August 2008, based on several veterinary and entomological reports of WNV circulation in northeastern Italy (9). The screen is performed on plasma specimens using 2 nucleic acid amplification technique (NAAT) tests (5, 7): the RT-PCR Cobas T/SCRN WNV USIVD test (Roche Molecular Diagnostics, Pleasanton, CA) and a transcription-mediated amplification (TMA) technique (Procleix Tigris WNV; Novartis Diagnostics, Siena, Italy).

Linnen and colleagues demonstrated that the Procleix assay has an overall specificity that exceeds 99.9% when fresh and frozen plasma specimens are used, despite the presence of potentially interfering substances, such as non-WNV viruses, bacterial and fungal contaminants, elevated levels of pathological or normal plasma proteins, and anticoagulants (8). Specifically, this method avoids the detection of human immunodeficiency virus (HIV) types 1 and 2; hepatitis A, B, and C viruses; and other flaviviruses from the JEV serogroup. The only virus that has generated false-positive reactions is Kunjin virus, which is genetically similar to WNV (6).

Recently, we and colleagues from Modena, Italy, described the first human cases of Usutu virus (USUV)-related illness in 2 immunocompromised patients (3, 10). In particular, we reported a subject who received an orthotopic liver transplantation (OLT) during USUV viremia and consequently developed neuroinvasive disease. USUV was detected in this patient’s blood, which was obtained during surgery, and no other viral infection, including WNV, was detected at that time. The liver donor was controlled for the presence of WNV and other organ-transmissible viral infections, including HIV, hepatitis C virus (HCV), and HBV, following national guidelines for the biosafety of organ donation (available online at http://www.trapianti.salute.gov.it/imgs/C_17_publicazioni_608_allegato.pdf).

Prior to these 2 reports, the involvement of USUV in the etiology of neurological disease in humans had not been documented (13).

The presence of WNV genome was determined in this OLT recipient in the routine context of the organ donation safety screen in plasma specimens by Procleix Tigris WNV assay. A plasma sample that was obtained at surgery, immediately before the OLT, was positive for WNV. The test was performed in triplicate and repeated twice, wherein 5 of 6 replicates had WNV-reactive values. The single analytical measurement for the Procleix Tigris WNV test comprised relative light unit (RLU) values between 285,308 and 165,815, wherein the signal/cutoff (S/CO) ranged from 5.62 to 3.26.

The same specimen was tested 3 times (individual samples) by Cobas T/SCRN WNV USIVD assay, each time generating a negative result.

Additional plasma samples from the recipient were obtained on days 1 and 3 post-OLT and evaluated first by Procleix Tigris WNV, generating RLU values of 92,240 and 594,545 and S/CO values of 1.66 and 10.80, respectively. Another plasma specimen was obtained from the patient on day 10 after OLT and was negative by Procleix Tigris WNV assay. All of these ancillary samples were evaluated by Cobas T/SCRN WNV USIVD assay, which deemed them all to be negative.

To examine the cause of the low levels of positivity by TMA
and explain the simultaneous negative results that were obtained with the Cobas assay, all plasma samples that were reactive by TMA were subjected to heminested PCR, which amplified the NS5 region of the Flavivirus genus. This method was developed to detect the principal pathogenic flaviviruses (including DENV, JEV, USUV, WNV, YFV, and Zika virus) by PCR for subsequent identification by sequencing (4).

This technique yielded a single amplicon of the expected size (220 bp), which was sequenced and analyzed by BLAST (http://www.ncbi.nlm.nih.gov/blast), showing 98% sequence identity to the genomes of USUV that had been isolated in Budapest, Hungary, and Vienna, Austria (available in GenBank under accession numbers EF206350.1 and AY453411.1, respectively) (2). Additional confirmation of USUV viremia was obtained by a USUV-specific PCR assay (12), and the virus was subsequently isolated in Vero E6 cells. These findings clearly suggest that the Procleix Tigris WNV assay is not intended to quantify the WNV genome in plasma samples. To obtain raw data on the number of copies of the USUV genome in our plasma specimens by this method, we prepared our specimens. To obtain raw data on the number of copies of the USUV genome in our plasma specimens, we prepared tants of Vero E6 cell cultures, ranging from 10⁰/H11002 up to 10⁹.

This series was subjected to the Procleix Tigris WNV assay, as reported above; only the first sample was positive (128,564 RLUs), suggesting that this TMA-based method detects this USUV isolate only when the viral concentration is at least 1 × 10⁰ TCID₅₀.

Our results confirm that the Procleix Tigris WNV assay is sensitive but that it can detect high loads of USUV in plasma specimens accidentally. Notably, USUV is genetically closer to JEV than to WNV (5). The false-positive results that were caused by USUV in the Procleix Tigris WNV assay must be considered in all of the areas in which this flavivirus circulates. In particular, due to the recent report of its ability to circulate in humans, further studies are required to define the actual risk of interhuman USUV transmission by blood transfusion or organ transplantation.

REFERENCES


