Absence of Neuroinvasive Disease in a Liver Transplant Recipient Who Acquired West Nile Virus (WNV) Infection from the Organ Donor and Who Received WNV Antibodies Prophylactically

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We describe the first case of West Nile virus infection in Europe with transmission from donor to recipient following liver transplantation. The infection was detected in the recipient 3 days after transplantation, during the asymptomatic phase. We also report an innovative prophylactic strategy based on infusion of West Nile virus hyperimmune plasma and gamma globulins that could be effective in preventing the appearance of a neuroinvasive disease.

West Nile virus (WNV) is an RNA arbovirus [1] transmitted by mosquitoes that acquire the virus from highly viremic birds and spread the infection to other species, including horses and humans [2]. WNV infection is generally seasonal, with activity peaks from mid-summer to late fall in temperate areas [1]. The incubation time in humans typically ranges from 2 days to 2 weeks, and ~80% of the infected subjects remain asymptomatic. The mildest expression of this infection is WNV fever, which manifests as mild influenza-like symptoms in ~20% of WNV-infected subjects [2]. The most severe clinical manifestation is the neuroinvasive disease, which is characterized by meningitis and encephalitis [2].

Since its discovery in the 1930s, WNV remained localized to certain areas of the Middle East, central Europe, Russia, and Africa [3]. In 1999, the virus appeared in wild birds in New York City, causing human cases of neuroinvasive disease; after this, WNV began to diffuse westward, throughout North America [4].

In Europe, the spread of WNV has been reported during the past 20 years in many countries [3]. In Italy, in past 2 years, WNV activity has been documented in the northeast [5, 6]. A recent serological survey of blood donors in this area reported a positivity ratio of 0.7% [7]. WNV transmission has also been associated with blood transfusion and solid-organ transplantation, and clinical studies indicate that organ transplant recipients are at high risk for neuroinvasive disease [2].

We describe the first case of WNV infection in Europe that was transmitted from a liver donor to the recipient, in whom infection was detected as early as 3 days after transplantation, during the asymptomatic phase; we also report an innovative prophylactic strategy, adopted to lower the risk of neuroinvasive disease, based on infusion of WNV hyperimmune plasma and immunoglobulin (Ig) according to the blood viral load.

Case report. The transplant donor was a 78-year-old woman who died from cerebral hemorrhage. She spent 2 weeks visiting relatives in the northeastern part of Italy, which is a WNV risk area. At that time, the donor was admitted to a local hospital for a cranial contusion, secondary to an accidental fall. At admission, she experienced fever (temperature, 39°C) and leukopenia (leukocyte count, 2500 cells/mL). Blood, bronchoalveolar lavage, and urine cultures were negative, and empirical antimicrobial therapy was started. After 2 days in the intensive care unit, she was declared brain-dead, her liver was harvested for transplantation, and no other organs were transplanted.

The day after the harvest (in September 2009), the Public Health Authority of the Emilia Romagna region, on the basis of data from the global surveillance network for WNV activity, mandated nucleic acid amplification testing (NAAT) for WNV in blood and organ donations from all subjects in WNV risk areas. Consequently, we retrospectively evaluated the donor’s blood sample for WNV by NAAT, which has a 95% limit of detection in individual testing of 40.3 target copies/mL (Cobas TAQ Screen WNV; Roche Diagnostics).

This technique was performed by the Clinical Microbiology Unit of the S. Orsola University Hospital in minipools of 6 samples. The minipool that contained the donor’s blood tested...
positive. The test was repeated twice with the single sample, and its positivity was confirmed. The donor plasma sample was tested further by transcription-mediated amplification, which has a 95% limit of detection in individual testing of 9.8 copies/mL (PROCLEIX WNV; Novartis Diagnostics), which confirmed the presence of the WNV genome.

The transplant recipient was a 25-year-old woman who had primary sclerosing cholangitis; she received her liver transplant in September 2009 at the Liver and Multiorgan Transplant Unit of S. Orsola University Hospital in Bologna. The postoperative immunosuppressive regimen was tacrolimus with scalar doses of steroids. The immediate postoperative period was uneventful, with the patient experiencing good functional recovery of the transplanted liver.

On the third postoperative day, the recipient tested positive for the presence of WNV genome by NAAT. To confirm that the WNV transmission was related to the organ transplantation, a blood specimen, collected from the recipient on the day before transplantation, was assayed by NAAT, which yielded a negative result and verified that the WNV infection was acquired from the donor through the transplanted organ. NAAT testing was performed every 3 days on plasma specimens obtained from the recipient patient until 45 days after transplantation (Figure 1).

The immunosuppressive therapy was promptly modified after the detection of WNV viremia—the tacrolimus blood level was reduced to a minimum, maintaining a trough of 5–7 ng/mL without any steroids. The patient’s immunosuppressive status was monitored regularly by Cylex Immuknow assay (Cylex), which measures the adenosine triphosphate activity in CD4 lymphocytes [8]. The Immuknow assay was performed every 3 days, and the dose of tacrolimus was adjusted to maintain a CD4 response of 130–450 ng/mL (Figure 1).

The day after the WNV genome was detected, immunological prophylaxis with fresh frozen plasma infusion, obtained from selected healthy WNV-seropositive blood donors, was also started (at 300–600 mL/day). These donors were selected among the individuals who tested positive in a recent serological survey [7] of healthy blood donors who lived in areas at risk for WNV infection. Each plasma sample was tested for WNV-specific antibody content by enzyme immunoassay (Euroimmun) and was further confirmed by immunofluorescence assay. Individual samples that had an IgG titer between 1:400 and 1:1600 were selected for plasma donation.

The plasma transfusion therapy was stopped on the 14th postoperative day, after 10 days of treatment, because of the inability to recruit additional WNV IgG-positive plasma units. For this reason, a commercially available immunoglobulin preparation that had high antibody titers against WNV, obtained from healthy Israeli blood donors (Omr-IgG-am; Omrix Biopharmaceuticals) [9], was used for 10 days under a compassionate-use protocol at a dose of 0.4 g/kg.

During this immunoprophylaxis, the patient did not develop any neurological complications, and a computed tomography scan of the encephalus and the spinal cord showed no abnormalities. Progressive increases in bilirubin and liver enzymes values were observed, with a concomitant increase in the Immuknow values, which peaked at 544 ng/mL (Figure 1). A liver biopsy revealed the presence of “moderate” acute rejection, which was treated immediately with 1 g of methylprednisolone, followed by rapid steroid tapering, to reach a minimum prednisone dose of 10 mg. As a consequence, the number of WNV

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**Figure 1.** Graphical representation of the West Nile virus viremia (viral load in copies/mL) (continuous line), Cylex ImmuKnow values (in ng/mL) (dashed line), and clinical events that occurred during the post-operative period. ACR, acute rejection; ACR-tp, acute rejection therapy.
genome copies in the patient's plasma increased sharply and rapidly. Nevertheless, the patient did not show any neurological signs related to WNV infection.

At 23 days after immunoglobulin prophylaxis was started, the patient developed a WNV IgM antibody response that increased from 1:400 to 1:1600 in the subsequent 10 days. When the IgM antibody titer reached 1:1600, the immunoprophylaxis was halted, after a total period of 33 days. A second episode of mild rejection occurred and was treated in the same way as was the first episode; in the second instance, there was no evidence of increased WNV replication in the patient's plasma.

The patient was finally discharged from the hospital on the 45th postoperative day. At present, the patient is well, has good liver function, and lacks any biochemical or neurological signs of WNV infection, which clearly suggests that our strategy, based on the modulation of immunosuppressive drugs, infusion of WNV-specific antibodies, and careful monitoring of the viral load, was effective in clearing WNV infection.

**Discussion.** This report describes the first case of a European patient who acquired a WNV infection though solid-organ transplantation, the early laboratory diagnosis of the infection, and the first prophylactic strategy that seems capable of reducing the risk of neuroinvasive WNV disease.

A previous report described the transmission of WNV from an infected organ donor to transplant recipients in the United States [10]. The WNV infection was detected in 4 recipients who received organs from a single donor who was infected via WNV viremic blood transfusion. Three of the recipients developed encephalitis, one of whom died. The fourth patient experienced only febrile illness without clinical signs of neurological involvement.

After this report, 3 additional cases of WNV infection were reported among 4 patients who received organs from a single donor, again in the United States [11]; 2 recipients developed neuroinvasive disease.

In all the cases of WNV infection following solid-organ transplantation reported to date, the infection in the recipients was suspected and confirmed only after the appearance of unexplained neurological illness or fever. Several recent articles have suggested that immunocompromised patients are apt to develop a severe form of WNV-related disease, accompanied by a greater incidence of neurological involvement and increased mortality [2, 12, 13]. Transplant recipients might have longer incubation periods and protracted viremia with late development of antibody, because of their immunosuppressive status [12]. Symptoms typically begin 7–17 days after transplantation, and antibodies develop after the period of highest viremia. In our patient, the WNV viremia was detected before any clinical evidence of infection, on the third postoperative day, and remained detectable for 35 days. An IgM antibody response specific to WNV developed on postsurgery day 23 and remained detectable until the patient's discharge.

Notably, the patient did not develop IgG seroconversion to WNV during hospital admittance or for >4 months after transplantation. This inability might be caused by persistent immunodepression or the continued availability of preformed IgG antibody during the viremic stage, which could have blocked the maturation of the immune response.

Today, no proven effective treatment or prophylaxis for WNV infection exists [14]; a randomized, placebo-controlled, double-blind trial of a plasma-derived IgG hyperimmune specific anti-WNV preparation (Omr-IgG-am) is underway [15]. In a recent article, 8 patients with WNV encephalitis were treated with high doses of intravenous immunoglobulin preparation, containing high titers of WNV-specific antibodies; significant improvements in symptoms were observed in 6 patients who received early treatment [9].

Animal studies suggested that WNV spreads to the brain during the very early stages of infection, despite the absence of clinical evidence of neurological impairment; in addition, the period in which immunoglobulin-based therapy has demonstrable efficacy in preventing the development of encephalitis is the first 4–6 days after infection [16]. In mice, treatment with WNV-specific immunoglobulin was more effective when administered during the viremic period, before the virus was detectable within the brain [17]. This finding emphasizes the importance of early diagnosis and preemptive treatment.

We believe that the decision to administer specific antibodies to our patient immediately after the detection of WNV viremia and to maintain this immunoglobulin-based prophylaxis until there was a complete clearance of the virus from the blood could have been important in preventing the spread of the infection throughout the central nervous system and any consequent development of neuroinvasive WNV-related disease.

We used an immunoglobulin preparation that contained a high titer of WNV antibodies at a dose of 0.4 g/kg for 10 days, because this daily dose has been recommended for the treatment of WNV encephalitis to prevent neuronal damage [18]. Furthermore, our strategy was to continue this immunoprophylaxis until a complete clearance of the virus was confirmed by 2 consecutive negative results of NAAT.

To minimize immunosuppression in the recipient, the therapy was reduced by modulating the dosage of immunosuppressive drugs to correlate with the results of the Immuknow assay. The importance of maintaining low levels of immunosuppression was confirmed by the sharp rise of WNV viremia that was observed after the administration of steroids for acute rejection during the third week after transplantation (Figure 1).

In conclusion, this is the first report of a liver transplant recipient who acquired a WNV infection from the donor for whom the laboratory diagnosis was obtained early enough to allow...
preemptive therapy with high titers of specific immunoglobulin, consistent reduction of immunosuppression therapy, and virological follow-up that effected an uneventful infection and might have prevented the spread of the virus throughout the central nervous system. It is important to underline that this case is the first case of transplantation in which WNV infection was noted before the appearance of clinical symptoms and it is consequently difficult to assess if and how the immunoprophylaxis could act to prevent the neurological involvement of the patient or if the viral load was too low to induce the neuroinvasive disease. Some recommendations can be proposed:

1. The importance of testing for the presence of WNV in organ donors during vector activity season in WNV risk areas
2. The possibility of preventing WNV neuroinvasive disease by treatment with WNV-specific antibodies, started before clinical onset (preemptive treatment)
3. The importance of virological follow-up of infected transplant recipients, using a quantitative test to define the blood viral load.
4. The need to modulate the patient’s immunosuppression with regard to viral load and rejection
5. The importance of continuing the preemptive antibody treatment until the virus is cleared from the blood.

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