Primary Versus Nonprimary West Nile Virus Infection: A Cohort Study

Galia Rahav,1,4,* Michal Hagin,1,4* Yasmin Maor,1,4* Gilad Yahalom,7 Musa Hindiyeh,2,4 Ella Mendelson,3,4* and Hanna Bin3,4*

1Infectious Diseases Unit, 2Department of Neurology, and 3Central Virology Laboratory, Ministry of Health, Chaim Sheba Medical Center, Tel Hashomer, and 4Sackler School of Medicine, Tel Aviv University, Israel

**Background.** Since 2001, we have observed patients with a clinical picture consistent with West Nile virus (WNV) infection, which was defined as nonprimary infection (NPI) owing to the presence of highly elevated serum immunoglobulin G antibody titers with a high avidity index (≥55%), absent or low titers of serum and cerebrospinal fluid (CSF) immunoglobulin M, and occasionally positive results of WNV-specific real-time reverse-transcription polymerase chain reaction analysis of CSF and/or blood specimens.

**Methods.** We investigated 124 patients with a diagnosis of primary WNV infection (PI) or NPI during 2005–2007 at Sheba Medical Center (Tel-Hashomer, Israel). Logistic regression was used to evaluate the association of variables with PI and NPI and with in-hospital mortality.

**Results.** A total of 68 and 50 patients with PI and NPI, respectively were included; 6 patients had incomplete data. In multivariate models, NPI was significantly associated with underlying psychiatric disorders (adjusted odds ratio [aOR], 13.73; 95% confidence interval [CI], 2.28–82.56; \( P = .004 \)), hospitalization during winter and spring (aOR, 8.82; 95% CI, 1.59–48.87; \( P = .013 \)), and fever (aOR, 0.61; 95% CI, .39–.95; \( P = .031 \)). In-hospital mortality was significantly associated with NPI (aOR, 3.86; 95% CI, 1.12–13.28; \( P = .032 \)) and a higher Charlson comorbidity index (aOR, 1.37; 95% CI, 1.03–1.83; \( P = .032 \)).

**Conclusions.** The possibility that NPI may be an emerging clinical entity with a high mortality rate must be considered seriously.

**Keywords.** West Nile virus; primary infection; nonprimary infection.

West Nile virus (WNV) is a neurotropic flavivirus that is transmitted to humans through the bite of infected mosquitoes during late summer and early fall [1]. Recently, WNV was found to be transmissible by blood transfusion and organ transplantation [2]. Most WNV-infected humans are asymptomatic, whereas approximately 20% of patients have flu-like symptoms. Less than 1% of infected patients develop West Nile neuroinvasive disease, characterized by aseptic meningitis, encephalitis, or acute flaccid paralysis, but the risk is higher among older persons and immunosuppressed individuals [1]. Transient viremia, which lasts for a week, occurs in most patients; however, in immunosuppressed patients it may last ≥2 months [3]. Viremia resolves with the appearance of WNV antibodies. Conventionally, the presence in humans of neutralizing antibodies is associated with protection from flavivirus infection [4].

Israel was first recognized as being endemic for WNV in 1940 [5]. During 2000, a major WNV outbreak occurred in Israel, with cases in 417 individuals, of whom 170 developed West Nile neuroinvasive disease and 35 died [6, 7]. During 2001–2004, 2006, and 2008–2009, the incidence decreased dramatically in sporadic cases in 10–50 people per year, of whom only 10 died. However, during 2005, 2006, and 2010, waves of WNV infection occurred, with 102, 163 and 114 cases, respectively (Central Virology Laboratory, Ministry of Health, Chaim Sheba Medical Center [SMC], Tel Hashomer, Israel, annual reports, unpublished data; Supplementary Table 1).

In March 2005, an 81-year-old man was admitted to the SMC with a 2-day history of fever, chills, and confusion. Findings of computed tomography of the head were unremarkable. Lumbar puncture demonstrated an opening pressure of 24 cm H2O, a cerebrospinal fluid (CSF) glucose level of 50 mg/dL, a CSF protein level of 72 mg/dL, and a CSF white blood cell count of 253 cells per high-power field, with 85% lymphocytes. Neither Gram stain nor culture revealed bacteria; polymerase chain reaction (PCR) analyses for herpes simplex virus (HSV), enteroviruses, and influenza virus were negative, as were tests for cryptococcal antigen, acid-fast bacilli (AFB), and human immunodeficiency virus (HIV). On the other hand, WNV-specific immunoglobulin G (IgG) titers measured in serum and CSF specimens 2 days following admission were high (15-fold above the standard cutoff). Tests for WNV-specific immunoglobulin M (IgM) in CSF and serum specimens were negative. WNV-specific
IgG avidity was 60%. WNV RNA was detected in CSF samples by real-time reverse transcriptase PCR (qRT-PCR) at $10^7$ copies/mL. The patient had no records of WNV infection in the past.

These findings led us to the present study, which investigated the epidemiology, natural history, and outcome of nonprimary WNV infection (NPI) and compared these findings to those for primary WNV infection (PI). As we were unable to differentiate between reactivation of previous infection with the same viral strain versus reinfection with a new strain or persistence of the same virus, we choose to define all these possibilities as NPI.

**MATERIALS AND METHODS**

The study was conducted following protocols approved by the SMC institutional review board. Since this was a retrospective observational study, written informed consent was not obtained from study subjects.

**Study Population**

The study population included all patients hospitalized with WNV infection at SMC, a tertiary university hospital with 1430 beds in central Israel, during 2005–2007. Patients who were treated with intravenous immunoglobulin 3 months prior to the diagnosis were excluded from the study.

**Definitions**

Clinical syndromes were classified as WNV encephalitis, indicated by fever, altered mental status, or other cortical signs (eg, seizures or focal neurologic signs); WNV meningitis was indicated by fever, meningeal signs, and abnormal CSF findings; WNV fever was indicated by flu-like symptoms without central nervous system (CNS) symptoms; acute flaccid paralysis with severe weakness of limbs or flaccid paralysis.

Confirmed PI was defined as a clinical picture consistent with WNV infection, with the following laboratory findings: (1) the development of anti-WNV IgG between the acute and convalescent phases of illness in serum or CSF, or (2) the presence of anti-WNV IgM in CSF [4], or (3) the detection of anti-WNV IgM and IgG in serum or CSF with low serum WNV-specific IgG avidity (<30%). Possible PI was defined when anti-WNV IgM was detected in the serum, with or without IgG.

NPI was defined as a clinical picture consistent with WNV infection with initial high levels of anti-WNV IgG (10-fold above the cutoff, or higher when performed during the first week of disease) and a high WNV-specific IgG avidity (≥55%) [8–10].

PI or NPI were further diagnosed as confirmed when WNV RNA was detected in serum or CSF, using qRT-PCR. All other recognized causes of documented encephalitis in Israel were ruled out.

**Laboratory Studies**

The National Center for Viral Zoonotic Infections at the SMC is the reference laboratory for arboviruses in Israel. Serological diagnosis of WNV infection was based on an IgM-capture enzyme-linked immunosorbent assay (MAC-ELISA) and an indirect IgG-WNV ELISA [11]. An IgG avidity of <30% indicated recent infection, while an avidity of ≥55% indicated NPI [8–10]. During the study period, WNV lineage 1 was the only recognized human pathogenic flavivirus in Israel. Other antigenically related flaviviruses, such as Usutu virus, Japanese encephalitis virus, and dengue virus, have not been detected in Israel, thus the likelihood of IgG ELISA cross-reactivity due to related flaviviruses was considered minimal. Israel does have related flaviviruses, such as Israel turkey encephalitis virus [12], but they are not recognized as human viruses.

Patient samples were analyzed for the presence of WNV RNA by a highly specific qRT-PCR assay, using primer sequences for the envelope gene, as previously described by Lanciotti et al and Shi et al [13, 14]. The primer-probe set detects lineage 1 WNV, which composes 97% of the mosquito-derived WNV strains detected in Israel and does not react with Usutu virus (Y. Lustig, Central Virology Laboratory, unpublished data). Briefly, viral RNA was extracted from 140 µL of patient CSF or serum samples, using the QIAamp Viral RNA Mini Kits (QIAGen, Hilden, Germany) in accordance with the manufacturer’s recommendations. Viral RNA was eluted from the column silica membranes in 50 µL elution buffer and stored at −70°C pending analysis. qRT-PCR was performed in triplicate by adding 10 µL of extracted patient samples to 40 µL of AgPath One-Step qRT-PCR Ambion Mastermix (Life Technologies) containing the WNV envelope gene primers and probes. The total qRT-PCR reaction volume was 50 µL. A patient’s sample was considered positive for WNV RNA if WNV RNA was detected by any of the 3 qRT-PCR reactions [15].

In patients with encephalitis, CSF specimens were collected for HSV-specific PCR analysis, enterovirus-specific PCR analysis, influenza virus–specific PCR, Epstein-Barr virus and cytomegalovirus-specific PCR, cryptococcal antigen analysis, and AFB stain and culture. HIV ELISA was also performed.

**Data Collection**

Demographic, epidemiologic, and clinical data were obtained by reviewing medical records. Hospitalizations were classified according to season. Comorbidity was divided into immunosuppression (ie, hematologic malignancy, immunosuppressive therapy or radiation in the preceding month, autoimmune disease, and AIDS), general comorbidity (ie, ischemic heart disease, congestive heart failure, diabetes mellitus, chronic renal failure, liver cirrhosis, and chronic lung disease), psychiatric comorbidity (ie, affective disorders, psychotic disorders, and anxiety disorders), and neurological comorbidity (ie, cerebrovascular disease, seizure disorder, dementia, brain tumor, and neurosurgical trauma). Charlson comorbidity index (CCI) and age-adjusted CCI were calculated as mean and median scores. CCI is the most widely used comorbidity index for prediction of 1-year mortality [16].
Statistical Methods

Data were collected using a web-based custom built program written in PHP, version 5.2.8, with MySQL, version 4.1.22. Univariate analysis was performed to assess variables associated with PI or NPI. Further analysis assessed risk factors associated with in-hospital mortality. The normality of interval variables was analyzed with the Shapiro–Wilk test. Comparison of groups regarding interval variables was conducted using the Student t test or the Mann–Whitney U test, as appropriate. The \( \chi^2 \) test or the Fisher exact test was used for categorical variables. A logistic regression model was constructed to evaluate the association of demographic, clinical, and laboratory variables with PI and NPI, as well as with in-hospital mortality. Five patients were removed from the sample because they were outliers: 2 were removed because of age (6 and 17 years), and 3 were removed because of their CCI (≥6). Stepwise backward elimination logistic regression models were performed. The probability was .05 for stepwise entry and .10 for stepwise removal.

Variables were selected for a multivariate regression analysis if, in univariate analysis, the \( P \) value was <.25 [17], data were available for ≥94% patients, and variable occurred in >10% of patients [6]. Variables were added or dropped on the basis of results of the likelihood ratio test. The variables age, immunosuppression, and encephalitis were introduced into the model for PI versus NPI, whereas the variables age, encephalitis, and CCI were entered into the mortality model. The \( c \) statistic was used to compare the goodness of fit of logistic regression models, values range from 0.5 to 1.0. Models are considered reasonable when the \( c \) statistic is higher than 0.7 and strong when \( c \) exceeds 0.8. Statistical analysis was conducted using SPSS software, version 20.0. Two-sided \( P \) values of <.05 were considered to indicate statistical significance.

RESULTS

The number of NPI increased in Israel from 1 patient in 2000 to 102 patients in 2010 (Supplementary Table 1). Between 2005 and 2007, 124 patients were hospitalized with WNV infection at SMC. Forty-four patients received a diagnosis of WNV infection in 2005, and WNV infection was diagnosed in 37 and 43 patients in 2006 and 2007, respectively. Seventy-one patients (57%) had WNV infection diagnosed as PI. A total of 65 patients had definite PI, while 6 had possible PI. Nine and 44 patients had definite and possible NPI, respectively (Supplementary Table 2). Six patients were excluded because their records lacked sufficient information (3 had PI and 3 had NPI).

Lumbar puncture was performed in 63% of patients overall (74 of 118 patients), including 65% (44 of 68 patients) in the PI group and 60% (30 of 50 patients) in the NPI group (\( P = .60 \)).

Primary WNV infection was diagnosed by WNV-specific IgG seroconversion in 33 patients, appearance of WNV-specific IgM in the CSF specimen in 24 patients, and detection of IgM and IgG in serum or CSF specimens with a low serum WNV-specific IgG avidity in 11 patients (Supplementary Table 2). Patients with PI had WNV-specific IgG titers 2–5-fold above the cutoff. Results of qRT-PCR analysis of CSF and serum specimens were positive in 3 patients and 1 patient, respectively. NPI was characterized by high levels of WNV-specific IgG (≥10-fold above the cutoff) in serum or CSF specimens and high WNV-specific IgG avidity in 50 patients. Results of qRT-PCR analysis of CSF and serum specimens were positive in 4 and 5 patients, respectively.

The median age was 70.0 years. Sex and age were not significantly associated with infection type (Table 1). PI occurred mainly during summer and autumn (66 of 68 cases [97%]), while 20% of NPI cases (10 of 50) occurred during winter and spring (\( P = .002 \)). Underlying diseases were diagnosed in 78% of patients (92 of 118), including 71% of patients (48 of 68) with PI and 88% (44 of 50) with NPI (\( P = .024 \); Table 1). The difference in age-adjusted CCI approached statistical significance (\( P = .063 \)), with a higher median score among patients with NPI. Underlying immunosuppression was diagnosed in 19% of patients. Psychiatric comorbidity was significantly more frequent among patients with NPI (20% vs 4%; \( P = .008 \)). The association between the type of psychiatric disorder (depressive vs psychotic) and type of infection (PI vs NPI) was not statistically significant. Preexisting neurological comorbidity occurred among 42% of patients, more frequently in patients with NPI (\( P = .006 \)). A relatively higher prevalence of a previous neurosurgical trauma was found in the NPI group (16% [8]), compared with the PI group (1% [1]; \( P = .004 \)).

Seventy-two patients (61%) had encephalitis diagnosed, 27 (23%) had West Nile fever diagnosed, 10 (9%) had acute flaccid paralysis diagnosed, and 9 (8%) had meningitis diagnosed (Table 1). Although acute flaccid paralysis occurred among 12% of patients with NPI and among 6% of patients with PI, the difference was not significant. A total of 88% of patients with NPI had a fever (temperature, ≥38°C) on admission, which was significantly higher than the frequency among patients with PI (Supplementary Table 3). Psychiatric disorder (new onset or exacerbation of a previously diagnosed disorder, lasting ≥24 hours) appeared in 6% (7 patients with NPI and 0 with PI; \( P = .040 \)). Five patients had depressive disorders (3 patients had newly diagnosed depression, and 2 had aggravation of previously diagnosed controlled depression). Two patients had schizophrenia: diagnosis in one was new, and in the other WNV infection was associated with aggravation of psychotic symptoms. Postural instability was detected at diagnosis in 4% and 16% of patients with PI and NPI, respectively (\( P = .050 \)). Nausea and vomiting occurred in 35% of patients and was significantly more frequent among patients with primary infection (\( P = .036 \)). Headache appeared among 39%, decline in the level of consciousness (lasting ≥24 hours) appeared in 36%, and cognitive decline appeared in 22%. Focal neurological signs occurred in 27%. Urinary incontinence developed...
among 21%. Seizure appeared in 14%. Rash developed in 9%.

No differences were detected between those with PI and those with NPI with regard to these clinical presentations (Supplementary Table 3).

Abnormal findings of liver function tests were more frequent among patients with NPI ($P = .05$).

Among 9 patients with definite NPI, 6 had encephalitis, 2 had acute flaccid paralysis, and 1 had West Nile fever. Six of the definite cases acquired WNV infection during winter, 2 acquired infection during summer, and 1 acquired infection during spring.

Factors associated with NPI were presence of an underlying psychiatric disorder (odds ratio [OR], 13.73; 95% confidence interval [CI], 2.28–82.56; $P = .004$), season of hospitalization (OR, 8.82; 95% CI, 1.59–48.87; $P = .013$), and fever (OR, 0.61; 95% CI, 0.39–0.95; $P = .031$; Table 2). Although acute flaccid paralysis occurred in 12% and 6% of patients with NPI and PI, respectively, this factor was not introduced to the multivariate analysis, owing to the small numbers. The $c$ statistic for the overall predictive value of the model was 0.794 (95% CI, 0.706–0.882; $P < .001$). The interactions of infection type and each of the variables included in the model were not significant.

The in-hospital mortality rate was 9% among patients with PI and 20% among patients with NPI ($P = .080$; Table 3).

### Table 1. Demographic Data, Comorbidities, and Diagnosis at Presentation Among Patients With Primary or Nonprimary West Nile Virus Infection

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Primary Infection (n = 68)</th>
<th>Nonprimary Infection (n = 50)</th>
<th>Overall (n = 118)</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y, median (range)</td>
<td>66.00 (7–90)</td>
<td>71.00 (16–93)</td>
<td>70.00 (7–93)</td>
<td>.43</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td>.53</td>
</tr>
<tr>
<td>Female % (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male % (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Season of hospitalization</td>
<td></td>
<td></td>
<td></td>
<td>.002</td>
</tr>
<tr>
<td>Summer and autumn</td>
<td>97 (66)</td>
<td>80 (40)</td>
<td>90 (106)</td>
<td></td>
</tr>
<tr>
<td>Winter and spring</td>
<td>3 (2)</td>
<td>20 (10)</td>
<td>10 (12)</td>
<td></td>
</tr>
<tr>
<td>Comorbidity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Charlson comorbidity index</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>3.99 ± 2.92</td>
<td>4.82 ± 2.56</td>
<td>4.34 ± 2.79</td>
<td>.063</td>
</tr>
<tr>
<td>Median (range)</td>
<td>4 (0–13)</td>
<td>5 (0–11)</td>
<td>4 (0–13)</td>
<td></td>
</tr>
<tr>
<td>Any underlying disease*</td>
<td>71 (48)</td>
<td>88 (44)</td>
<td>78.0 (92)</td>
<td>.024</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>32 (22)</td>
<td>38 (19)</td>
<td>35 (41)</td>
<td>.52</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>24 (16)</td>
<td>28 (14)</td>
<td>26 (30)</td>
<td>.69</td>
</tr>
<tr>
<td>Chronic renal failure</td>
<td>13 (9)</td>
<td>12 (6)</td>
<td>13 (15)</td>
<td>.76</td>
</tr>
<tr>
<td>Immunosuppression*</td>
<td>16 (11)</td>
<td>22 (11)</td>
<td>19 (22)</td>
<td>.42</td>
</tr>
<tr>
<td>Psychiatric comorbidity*</td>
<td>4 (3)</td>
<td>20 (10)</td>
<td>11 (13)</td>
<td>.008</td>
</tr>
<tr>
<td>Neurologic comorbidity*</td>
<td>31 (21)</td>
<td>56 (28)</td>
<td>42 (49)</td>
<td>.006</td>
</tr>
<tr>
<td>Diagnosis at presentation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Encephalitis</td>
<td>60 (41)</td>
<td>62 (31)</td>
<td>61 (72)</td>
<td>.85</td>
</tr>
<tr>
<td>West Nile fever</td>
<td>26 (18)</td>
<td>18 (9)</td>
<td>23 (27)</td>
<td>.28</td>
</tr>
<tr>
<td>Acute flaccid paralysis</td>
<td>6 (4)</td>
<td>12 (6)</td>
<td>9 (10)</td>
<td>.32</td>
</tr>
<tr>
<td>Meningitis</td>
<td>10 (7)</td>
<td>4 (2)</td>
<td>8 (9)</td>
<td>.30</td>
</tr>
</tbody>
</table>

Data are % (n) of patients, unless otherwise indicated.

*Diabetes mellitus (n = 41), ischemic heart disease (n = 30), chronic renal failure (n = 15), solid malignancy (n = 11), congestive heart failure (n = 8), chronic lung disease (n = 8), peripheral vascular disease (n = 4), peptic ulcer disease (n = 2), and liver cirrhosis (n = 1). Immune compromising condition; psychiatric disorder and neurologic comorbidity.

* Treatment with immunosuppressive drugs (n = 12) or radiation (n = 1) in the preceding month, hematologic malignancy (n = 9), autoimmune disease (n = 9), and nephrotic syndrome (n = 1).

* Depressive disorder (n = 7), schizophrenia (n = 3), other psychotic disorder (n = 2), and schizoaffective disorder (n = 1).

* Neurologic comorbidity: dementia (n = 24), cerebrovascular disease (n = 22), neurosurgical trauma (n = 9), brain tumor (n = 6), seizure disorder (n = 3), spinal compression or stenosis (n = 3) and other neurologic disease (n = 7).

### Table 2. Factors at Admission Associated With Nonprimary West Nile Virus Infection

<table>
<thead>
<tr>
<th>Variable</th>
<th>$P$ Value</th>
<th>aOR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psychiatric comorbidity</td>
<td>.008</td>
<td>13.73 (2.28–82.56)</td>
</tr>
<tr>
<td>Season (winter or spring)</td>
<td>.002</td>
<td>8.82 (1.59–48.87)</td>
</tr>
<tr>
<td>Fever</td>
<td>.036</td>
<td>0.61 (0.39–0.95)</td>
</tr>
<tr>
<td>Abnormal result of LFT</td>
<td>.006</td>
<td>3.13 (0.99–10.91)</td>
</tr>
<tr>
<td>Age (per y increase)*</td>
<td>.43</td>
<td>1.03 (0.99–1.06)</td>
</tr>
<tr>
<td>Charlson comorbidity index*</td>
<td>.12</td>
<td>1.26 (0.95–1.69)</td>
</tr>
<tr>
<td>Encephalitis*</td>
<td>.85</td>
<td>0.21 (0.83–1.71)</td>
</tr>
<tr>
<td>Immunosuppression*</td>
<td>.42</td>
<td>1.35 (0.43–4.29)</td>
</tr>
</tbody>
</table>

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; LFT, liver function test.

Data are % (n) of patients, unless otherwise indicated.

* Variable was forced into the model.

* Data are per unit increase in index.
Neurological sequelae at the time of discharge were more common in patients with PI, although the difference was not statistically significant. The median duration of hospitalization was similar in both groups.

In a multivariate regression model, in-hospital mortality was found to be significantly associated with NPI (OR, 3.86; 95% CI, 1.12–13.28; \( P = .032 \)) and CCI (OR, 1.37; 1.03–1.83; \( P = .032 \)), (Table 4). The \( c \) statistic for the overall predictive value of the model was 0.826 (95% CI, .718–.934; \( P < .001 \)).

### DISCUSSION

In this study, we describe patients with NPI characterized by a typical clinical presentation, highly elevated anti-WNV IgG titers compatible with secondary infection and high IgG-avidity; WNV RNA was detected in serum or CSF specimens from 17% of cases. Substantial efforts were made to rule out other compatible diagnoses. Furthermore, no other endemic cross-reacting pathogenic flavivirus have been shown to infect humans in Israel.

Recurrent CNS syndromes occur during neurotropic flavivirus infections [18]. Several reports have documented the occurrence of symptomatic Japanese encephalitis virus infection in patients with preexisting immunity [19–21]. Recurrent encephalitic syndromes from tick-borne encephalitis flaviviruses were also observed in 3 patients after complete recovery [22]. Recurrent infections are common in dengue hemorrhagic fever. WNV infection can cause persistent infection in animal models [23–27]. Recurrent infection due to WNV was recently diagnosed in 4 cases that presented with limb weakness [28]. WNV RNA was detected in 20% of urine samples collected from convalescent patients 1.6–6.7 years after WNV infection [29]. Two publications described the persistence of WNV nucleic acid in whole-blood specimens months after clearance in plasma [30, 31]. This persistent virus infection can cause progressive symptoms or remain silent until reactivation occurs.

All patients in our study had a clinical presentation compatible with WNV infection. The laboratory diagnosis of West Nile neuroinvasive disease is typically made by detecting the presence of WNV-specific IgM in CSF [4, 32]. If CSF is not available, then serum can be tested; IgG seroconversion is a well-established criterion for the definition of PI; detection of IgM with or without detection of IgG in a serum sample needs to be interpreted cautiously when diagnosing acute infection, since IgM antibodies can persist for extended periods. Avidity assays have been used to differentiate between acute infection or PI and recurrent or reactivated disease in a number of infections, such as cytomegalovirus infection, toxoplasmosis, rubella, and tick-borne encephalitis [8, 33–35]. Recently, WNV-specific IgG avidity was found as a contributory laboratory tool to differentiate between recent and past exposure to WNV [9, 10]; 11 of 68 patients in our study were classified as having PI, using the low avidity criteria. Although previous studies defined WNV infection as detection of IgM in a CSF or serum specimen [6, 7], in this study only 5 of 68 patients with PI had IgM detected in sera as the sole evidence of acute infection and were defined as possibly having PI. Although viremia is present especially during the first 4 days of illness, WNV has rarely been isolated from the serum or CSF specimens from patients prior to the development of antibody response [15]. A number of molecular amplification assays with sensitivities surpassing that of virus isolation of detection of WNV have been reported, but these are also of limited usefulness in human diagnostics, owing to the low magnitude and transient nature of viremia. In a study of patients with serologically confirmed acute West Nile meningoencephalitis, the sensitivity of TaqMan RT-PCR for detecting

### Table 3. Outcome in Patients With Primary or Nonprimary West Nile Virus Infection

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Primary Infection (n = 68)</th>
<th>Nonprimary Infection (n = 50)</th>
<th>Overall</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>In-hospital mortality</td>
<td>9 (6)</td>
<td>20 (10)</td>
<td>14 (16)</td>
<td>.08</td>
</tr>
<tr>
<td>Neurological sequelae</td>
<td>41 (28)</td>
<td>32 (16)</td>
<td>37 (44)</td>
<td>.30</td>
</tr>
<tr>
<td>Cognitive decline</td>
<td>23 (16)</td>
<td>20 (10)</td>
<td>22 (26)</td>
<td>.65</td>
</tr>
<tr>
<td>Length of stay, d</td>
<td>9.0 (1–244)</td>
<td>10.5 (2–132)</td>
<td>9.5 (1–244)</td>
<td>.96</td>
</tr>
</tbody>
</table>

Data are % (n) of patients or median value (range).

### Table 4. Univariate and Multivariate Regression Analysis of Factors Associated With In-Hospital Mortality

<table>
<thead>
<tr>
<th>Variable</th>
<th>Survived (n = 102)</th>
<th>Died (n = 16)</th>
<th>( P ) Value</th>
<th>aOR (95% CI)</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonprimary infection</td>
<td>39.2</td>
<td>62.5</td>
<td>.08</td>
<td>3.86 (1.12–13.28)</td>
<td>.032</td>
</tr>
<tr>
<td>Charlson comorbidity index*</td>
<td>2.0 (0–7)</td>
<td>3.0 (0–9)</td>
<td>.07</td>
<td>1.37 (1.03–1.83)</td>
<td>.032</td>
</tr>
<tr>
<td>Encephalitis*</td>
<td>55.9</td>
<td>93.8</td>
<td>.004</td>
<td>7.62 (.90–64.88)</td>
<td>.063</td>
</tr>
<tr>
<td>Maximum temperature, °C</td>
<td>39.0 (36.3–40.7)</td>
<td>39.4 (37.5–41.0)</td>
<td>.07</td>
<td>1.70 (.91–3.16)</td>
<td>.094</td>
</tr>
<tr>
<td>Age, y*</td>
<td>70.0 (7–93)</td>
<td>75.0 (16–84)</td>
<td>.14</td>
<td>1.05 (.96–1.05)</td>
<td>.858</td>
</tr>
</tbody>
</table>

Data are % (n) of patients or median value (range).

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval

*a* Variable was forced into the model.

*b* Per unit increase in index.

*c* Per y increase in age.
WNV nucleic acids in acute-phase CSF and serum specimens was 57% and 14%, respectively [13]. qRT-PCR results were positive in our study in only 4 patients with PI.

The phenomenon of increased antibody titers in NPIs is also documented with measles virus in subacute sclerosing panencephalitis cases and in Dengue hemorrhagic fever. The presence of high antibody titers could have an immunological consequence, such as exacerbating disease severity in the form of antibody-dependent enhancement, in which infection of Fc receptor–bearing cells, usually macrophages, is increased by the presence of antiviral antibodies. This enhancement is usually seen as an increase in the yield of infectious virus from infected cells. Antibody-dependent enhancement was demonstrated in vivo in mice treated with monoclonal antibodies against yellow fever [36] and was suggested as the mechanism responsible for the severity of dengue hemorrhagic fever [37].

Since the last epidemic of WNV in Israel, during 2000, the number of patients with NPI has increased dramatically, perhaps due to a relatively high prevalence of WNV seropositivity.

WNV morbidity is mainly recorded in Israel during the late summer and fall, when mosquito-associated infections occur [38]. The probability that patients who presented with WNV disease during the winter or spring will have an infection caused by a mosquito bite is very low. It seems that infections in the 20% with NPI who presented during the winter and spring represent reactivation of a persistent virus and that all other patients with NPI could have reactivation or persistence of the virus. Patients with NPI had a significantly higher frequency of underlying psychiatric and neurological comorbidities. The significant underlying psychiatric morbidity on admission could be due to persistent WNV infection of the brain. WNV infection may precipitate depression or schizophrenia or be able to trigger these psychiatric morbidities in older adults. Nolan et al. conducted a longitudinal study to assess mental health outcomes among a cohort of 171 WNV-positive patients in Houston, Texas, over an 8-year period. They found that 35% of their participants had new-onset depression [39].

The mortality rate among patients with NPI was higher (20% vs 8.8%), probably reflecting the higher comorbidity burden in this group. The frequency of neurological sequelae was similar in both groups. Using logistic regression, we found that NPI was associated with increased rates of out-of-season hospitalization, the presence of an underlying psychiatric disorder, and a lower degree of fever. In-hospital mortality was associated with NPI and CCI.

Persistent virus or reactivation of low-level persistent virus is better suitable to the study population, as it occurs during the winter and spring; furthermore, persistent virus in the CNS would result in worse neurological and psychological outcomes, which could be triggered by immunosuppression, aging-related conditions, or underlying conditions. Reinfecion by a different lineage is less likely due to the absence of mosquitoes during the winter and spring. Furthermore, lineage 2 was found only in <3% of mosquito pools (3 of 102) tested in Israel and only in 2009 and 2010. Thus, this lineage, which is very rare in Israel and is not present every season, is not likely to cause all of the cases described here. We also have not found lineage 2 in patients, even after including a primer and probe set directed to the NC region, which detects both lineage 1 and 2, together with the Env primer-probe set (unpublished data).

The study has several limitations: first, the definition of NPI has not yet been fully validated, but the combination of a classical clinical picture, together with very high titers of WNV-specific IgG and high avidity, strongly support the diagnosis of NPI. Ruling out all other compatible clinical diagnoses and finally detecting WNV RNA in CSF and serum samples from some patients improved the validity of the diagnosis of NPI. Second, sequential CSF and serum samples were not available for all patients. Third, we used a conventional logistic regression method, which is appropriate for large samples. As only 16 patients died in the study, it is possible that exact logistic regression methods fit the model better. Although King and Zeng accurately described the problem and proposed an appropriate solution, there are still a lot of misconceptions about this issue [40]. Finally, we recognize the difficulties of ruling out persistent low-level activity of WNV, rather than reactivation or repeated mosquito-delivered infections. However, this will require larger numbers of patients for more-detailed analyses before we can understand precisely what is happening. Nevertheless, the possibility that NPI may be an emerging clinical entity with a high mortality rate must be considered seriously.

Supplementary Data
Supplementary materials are available at http://jid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Note
Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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