Hospital-based enhanced surveillance for West Nile virus neuroinvasive disease

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SUMMARY

Accurate data on the incidence of West Nile virus (WNV) disease are important for directing public health education and control activities. The objective of this project was to assess the underdiagnosis of WNV neuroinvasive disease through laboratory testing of patients with suspected viral meningitis or encephalitis at selected hospitals serving WNV-endemic regions in three states. Of the 279 patients with cerebrospinal fluid (CSF) specimens tested for WNV immunoglobulin M (IgM) antibodies, 258 (92%) were negative, 19 (7%) were positive, and two (1%) had equivocal results. Overall, 63% (12/19) of patients with WNV IgM-positive CSF had WNV IgM testing ordered by their attending physician. Seven (37%) cases would not have been identified as probable WNV infections without the further testing conducted through this project. These findings indicate that over a third of WNV infections in patients with clinically compatible neurological illness might be undiagnosed due to either lack of testing or inappropriate testing, leading to substantial underestimates of WNV neuroinvasive disease burden. Efforts should be made to educate healthcare providers and laboratorians about the local epidemiology of arboviral diseases and the optimal tests to be used in different clinical situations.

Key words: Arbovirus, encephalitis, meningitis, surveillance, West Nile virus.

INTRODUCTION

West Nile virus (WNV) is the leading cause of arthropod-borne viral (arboviral) disease in the continental United States [1]. Although the majority of persons infected with WNV remain asymptomatic, about 20% develop an acute systemic febrile illness and <1% develop neuroinvasive disease, which typically manifests as meningitis, encephalitis, or acute flaccid paralysis [2, 3]. Detection and reporting of WNV neuroinvasive disease cases is generally assumed to be more consistent and complete than that for non-neuroinvasive disease cases and is often used to estimate total burden of infection [4].

Two previous studies described WNV underreporting in the United States, but they did not examine the completeness of WNV testing in patients with clinically compatible illnesses [5, 6]. During an outbreak

of WNV disease in Arizona, a review of medical records from patients with meningitis or encephalitis determined that only 40% were tested for WNV [7]. In that investigation, younger patients and those with less severe disease were less likely to have WNV testing performed. However, the proportion of patients who were not tested but were infected with WNV was unknown. The objective of this project was to assess underdiagnosis of WNV neuroinvasive disease through laboratory testing of patients with suspected viral meningitis or encephalitis at selected hospitals serving WNV-endemic regions of Arizona, Minnesota, and California from 2011 to 2013.

METHODS

Surveillance locations

A convenience sample of hospitals from areas with higher incidence of WNV disease in the three states was selected for participation in this enhanced surveillance project. Not all hospitals participated in each year. In 2011, three hospitals participated in the project, including: UC Davis Medical Center in Sacramento County, California; Essentia Health-St Mary's Medical Center in St Louis County, Minnesota; and Rice Memorial Hospital in Kandiyohi County, Minnesota. In 2012, four additional hospitals participated, including: Banner Desert Medical Center and Banner Estrella Medical Center in Maricopa County, Arizona; Eisenhower Medical Center in Riverside County, California; and Sutter Medical Foundation in Sacramento County, California. In 2013, one new hospital participated (Banner Good Samaritan in Maricopa County, Arizona) and none of the California hospitals participated that year.

Case definition and specimen testing

Patients were considered to be a suspect case of viral meningitis or encephalitis if they had a cerebrospinal fluid (CSF) specimen with pleocytosis [⩾5 white blood cells (WBC)/ml] collected during the known arboviral transmission season. The arboviral transmission season was defined separately for each state based on regional epidemiological data, but included at least 1 June to 31 October at each site. Patients with known non-arboviral infectious aetiologies (e.g. herpes simplex virus, enterovirus, *Cryptococcus, Neisseria meningitidis, Streptococcus pneumoniae*, or *Haemophilus influenza*) or non-infectious aetiologies (e.g. stroke,

leukaemia involving the central nervous system, vasculitis, or toxic encephalopathy) identified through testing ordered by the clinical care team were excluded.

All CSF specimens were tested for immunoglobulin M (IgM) antibodies against WNV by enzyme-linked immunosorbent assay (ELISA), either at the state public health laboratory according to the methods of the enhanced surveillance project and/or a commercial laboratory if ordered by the attending physician. Some specimens with positive IgM antibody results were sent to the Centers for Disease Control and Prevention Arboviral Diseases Diagnostic Laboratory for confirmation via plaque-reduction neutralization testing (PRNT). In some states, additional testing for other neuroinvasive arboviral infections was performed, including flavivirus reverse transcription–polymerase chain reaction (RT–PCR) with sequencing of positive results, and ELISA or indirect immunofluorescent assay for IgM antibodies against eastern equine encephalitis, California serogroup, Powassan, St Louis encephalitis, or western equine encephalitis viruses. Aliquots for this surveillance project were only made if sufficient specimen was available after all other tests ordered by the attending physician had been completed. If there was no residual specimen remaining from the original CSF draw, no additional specimen was collected and limited demographic information was obtained. Some patients that met the study criteria for suspect viral meningitis or encephalitis had serum specimens tested for WNV IgM antibodies. Age, sex, and county of residence were collected on patients meeting the inclusion criteria. In addition, CSF WBC counts were collected where available; in some situations, specimens were identified as having pleocytosis according to inclusion criteria, but the actual WBC value was not recorded in the project database. For cases testing positive for WNV through the enhanced surveillance project, information was also obtained on any commercial WNV testing performed. The proposal for this project was reviewed by human subjects' advisors from participating health departments and determined to be public health surveillance and not research; therefore, the project was exempt from Institutional Review Board review.

RESULTS

A total of 337 patients met the study criteria for suspect viral meningitis or encephalitis. Of these, 279

Table 1. *Age and sex of suspected viral meningitis and encephalitis patients by West Nile virus (WNV) immunoglobulin M (IgM) antibody results in cerebrospinal fluid*

	WNV IgM-positive (N = 19)		WNV IgM-negative (N = 260)		
	No.	(%)	No.	(%)	P value
Age group, years					
<20	0	(0)	83	(32)	<0·01
20–39	5	(26)	83	(32)	
40–69	8	(42)	72	(28)	
⩾70	6	(32)	22	(8)	
Sex*					
Male	10	(53)	117	(45)	0·53
Female	9	(47)	142	(55)	
CSF WBC†					
5–19	2	(11)	39	(15)	
20–34	3	(16)	21	(8)	0·06
35–49	2	(11)	3	(1)	
⩾50	3	(16)	41	(16)	
Unknown	9	(47)	156	(60)	

* Sex unknown for one patient.
† Cerebrospinal fluid white blood cells/ml.

(83%) had WNV IgM antibody testing performed on CSF; the remainder were not tested due to insufficient volume of sample. Patient's age, sex, and CSF WBC count were available for 28 (48%) of the 58 eligible samples that did not have testing performed. The median ages of tested [33, interquartile range (IQR) 14–50 years] and untested (35, IQR 6–46 years) patients were similar ($P = 0.63$). A larger percentage of untested patients were female (57%) than those tested (46%), but the difference was not statistically significant ($P = 0.77$). The median CSF WBC counts of tested (28, IQR 11–108) and untested (30, IQR 8–122) patients were also similar ($P = 0.81$).

Of the 279 CSF samples tested for WNV IgM antibodies, 258 (92%) were negative, 19 (7%) were positive, and two (1%) had equivocal results. The proportion of specimens testing positive of those submitted in a year was highest in 2013 (9/66, 14%), and was similar in 2011 (2/48, 4%) and 2012 (8/165, 5%). The proportion positive also varied by state; Minnesota (13%) had the highest proportion positive, followed by Arizona (6%) and California (5%). Patients with WNV IgM-positive CSF results were older (median 58, IQR 37–73 years) than those with negative results (median 32, IQR 13–49 years) ($P < 0.01$), but the two groups did not differ significantly by sex (Table 1). CSF WBC counts were available for 104 (40%) of the 260 IgM-negative specimens and 10 (53%)

of the 19 IgM-positive specimens. WBC counts were not significantly different between the IgM-positive (median 37, IQR 23–218) and IgM-negative (median 28, IQR 11–106) specimens ($P = 0.37$).

Of the 19 patients with WNV IgM-positive CSF specimens, 12 (63%) had WNV IgM testing ordered by the attending physician and were subsequently reported to the appropriate public health authorities. Of the remaining seven (37%) patients with WNV IgM-positive CSF specimens; four had WNV tested by RT–PCR ordered by their physician but all were negative and three had no WNV testing ordered by their physician.

Of all CSF samples tested for WNV IgM antibodies, 30 were also tested by WNV RT–PCR at a state health department. Only one (3%) of the CSF samples tested positive for WNV RNA by RT–PCR; the same sample had tested equivocal for WNV IgM antibodies. None of the other specimens were positive for WNV RNA (including four specimens that were positive for WNV IgM antibodies, one that was WNV IgM equivocal, and 24 that were negative for IgM antibodies).

Forty-four (16%) of the 279 specimens were tested for St Louis encephalitis virus IgM antibodies; none were positive. Twenty-nine (10%) of the 279 specimens were tested for Powassan virus IgM antibodies. One of these was positive for both Powassan virus and WNV

IgM antibodies; PRNTs performed on this specimen found a titre of 8 to Powassan virus and <4 to WNV, suggesting a recent Powassan virus infection. No other Powassan virus infections were identified. Nine (3%) of the 279 specimens were tested for eastern equine encephalitis, California serogroup, and western equine encephalitis virus IgM antibodies. One specimen was California serogroup virus IgM antibody-positive; PRNT performed on this specimen identified Jamestown Canyon virus infection. The patients with Powassan and Jamestown Canyon infections were Minnesota residents.

Thirty-eight patients that met the study criteria for suspect viral meningitis or encephalitis had serum specimens tested for WNV IgM antibodies. Of those, five were WNV IgM-positive and 33 were negative. Twenty-six of these patients also had CSF tested for WNV IgM antibodies; the remainder did not have sufficient volume of CSF for testing. Twenty-one patients were WNV IgM-negative on both serum and CSF. Three patients were WNV IgM-positive on both serum and CSF. One patient whose serum was collected 15 days after illness onset tested IgM-positive and their CSF sample collected 2 days after illness onset was IgM equivocal; however, the CSF for this patient was RT–PCR positive for WNV. One patient with serum and CSF collected 19 days after illness onset was serum IgM-positive and CSF IgM-negative; additional confirmatory testing of the serum with PRNT was negative.

DISCUSSION

To our knowledge, this is the first published report to assess underdiagnosis of WNV neuroinvasive disease through laboratory testing of patients with suspected viral meningitis or encephalitis in the United States. Overall, we found that 84% of patients with WNV IgM-positive CSF had WNV testing ordered by their attending physician. However, of those that were tested, 25% did not have the optimal testing needed to identify the WNV infection. These results suggest that over a third of WNV infections in patients with CSF pleocytosis might be undiagnosed due to either lack of testing or inappropriate testing, leading to substantial underestimates of WNV neuroinvasive disease burden.

We found that clinicians tended to test patients for WNV infection appropriately but do not always order the most appropriate tests. A review of medical records from patients with meningitis or encephalitis during an outbreak of WNV disease in Arizona showed that older patients and those with more severe disease were more likely to be tested [7]. The findings of this project support targeted testing of older persons, as those with WNV IgM-positive CSF were older than those with negative WNV testing. Available data for a subset of patients included in this study suggests that CSF WBC counts are not markedly different between those with positive and negative WNV testing. This finding is consistent with other studies and our current testing recommendations which do not suggest using this metric to determine which samples to test for WNV [8–10]. Furthermore, testing of CSF samples from all patients with aseptic meningitis or encephalitis and pleocytosis, as was performed in this study, is relatively labour intensive and the yield varied annually and between states. This variation in testing yield in patients with fever, aseptic meningitis, and meningoencephalitis during arboviral transmission season was also noted in Italy, where 30 (25%) of 120 samples tested positive for Toscana virus infection but none (0%) tested positive for WNV infection despite the presence of the virus in an adjoining area [11].

In this study, four (21%) of 19 patients with WNV IgM-positive CSF specimens had only WNV RT–PCR testing ordered as part of their routine clinical care and all were negative. Within the first few days of illness, WNV RNA may be detected in CSF or serum using RT–PCR [12]. However, the likelihood of detection using this method is relatively low as viral RNA is often absent by the time of symptom onset [13]. Of the 30 samples that underwent RT–PCR testing in a state public health laboratory, only one was positive. However, four of the RT–PCR negative samples were WNV IgM-positive. Limited data suggest that molecular testing may be more useful in selected situations such as in immunocompromised patients [14].

The presence of WNV-specific IgM antibodies in blood or CSF provides good evidence of recent infection but may also result from cross-reactive antibodies after infection with other flaviviruses or from non-specific reactivity. Whenever possible, positive IgM results should be confirmed by neutralizing antibody testing of acute- and convalescent-phase serum specimens at a state public health laboratory or CDC. The importance of this additional confirmatory testing was highlighted by the patient in this study who was determined to have confirmed Powassan virus infection by PRNT testing despite positive WNV IgM antibody results and the patient with WNV IgM-positive serum that failed to confirm as a true WNV infection.

The findings of this project are subject to several limitations. First, the enhanced surveillance was conducted in larger hospitals serving endemic areas within participating states. Testing practices in these facilities may have differed from other smaller facilities or facilities in areas where WNV infections are less common. In addition, the changes in participating hospitals throughout the study period resulted in variations in the physician population, which likely impacted testing practices over time. Second, data on the timing of specimen collection relative to illness onset were often not available. Therefore, it was not possible to determine what type of testing (serology *vs.* RT–PCR) would have been most appropriate or if specimens might have been collected too early in the course of the illness for antibody detection. Third, performing PRNT to confirm the IgM results and to determine the specific infecting flavivirus was not routinely completed for the IgM-positive specimens. Without performing PRNTs, it is not possible to know if WNV IgM positives reflect true WNV positives. Fourth, there was a large proportion of missing data for age, sex, and WBC counts for patients that were not tested for WNV due to a lack of available sample; therefore, the comparison of tested and untested patients may not be reliable. That said, it is unlikely that patients with missing data would be systematically different than those with available data. Finally, no clinical information and only limited CSF counts were available on patients included in the study. Medical records were reviewed at each of the institutions to ensure that patients met the inclusion criteria but those data were not uniformly collected and reported. Therefore, it was not possible to determine if there were differences in clinical presentation and signs in those tested and not tested or those with positive and negative WNV test results.

Our findings indicate that there is some degree of underdiagnosis of WNV infections in patients with clinically compatible neurological illness. However, systematically testing all CSF specimens with pleocytosis for WNV would require additional resources including testing beyond IgM ELISA to definitively confirm and rule out infections. Therefore, this approach is probably not sustainable for routine public health surveillance. Instead, efforts should be made to educate healthcare providers and laboratorians about the local epidemiology of arboviral disease and the optimal tests to use based on the underlying health status of the patient and the timing of specimen collection relative to illness onset.

DECLARATION OF INTEREST

None.

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