SHORT REPORT

Maculopapular rash and tremor are associated with West Nile fever and neurological syndromes

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J Neurol Neurosurg Psychiatry 2007;**78**:529–531. doi: 10.1136/jnnp.2006.107862

The clinical features associated with West Nile virus (WNV) infections are described based on data collected from history forms submitted with samples during a province-wide WNV testing programme. Age 40–59 years (OR 1.7, $p<0.008$), residence in the southeast of Alberta (OR 4.2, $p<0.001$), maculopapular rash (OR 8.6, $p<0.001$) or tremor (OR 3.6, $p<0.001$) were independently associated with WNV infection.

A number of case series describing the clinical features associated with West Nile virus (WNV) infections have been published.^{1–3} In these cases, neuromuscular weakness has been identified as a common feature. Most of these reports have included small numbers of patients, and have included only those with neurological illnesses. It is widely recognised that most patients infected by WNV are asymptomatic or have mild non-neurological illnesses, but large-scale studies covering the full disease spectrum are lacking.

In 2003, WNV testing was implemented at the Provincial Laboratory for Public Health (ProvLab, Calgary Health Region, Calgary, Alberta, Canada) to provide diagnostic testing for the province of Alberta, Canada. Pretest clinical information was sought from all doctors submitting samples for testing to assist with test triage and interpretation. This testing and surveillance infrastructure, which included clinical information on both cases and non-cases, facilitated a population-based review of the clinical features associated with WNV infection.

METHODS

Patient population, specimen and data collection

During the first season of WNV activity in the province of Alberta (1 June–30 November 2003), all WNV diagnostic testing for symptomatic patients and follow-up testing of blood donors identified as WNV RNA positive by Canadian Blood Services were performed at ProvLab. This public health laboratory serves a population of approximately three million people spread over a wide geographic area (255 000 square miles), with two major urban centres (Calgary and Edmonton). Before the onset of the WNV season, doctors were instructed to collect both a serum sample for WNV serology and a plasma sample for nucleic acid amplification testing (NAAT) from all patients with suspected WNV infection. Clinical history forms that included demographic data, date of onset of symptoms and a symptom checklist were also submitted with each set of samples. When specimens were received without a history form, a blank form was faxed to the doctor.

Serological testing of serum samples for WNV IgM

Serum samples were screened for WNV IgM using two commercial enzyme immunoassay (EIA) kits (West Nile Virus IgM Capture EIA, Focus Technologies, Cypress, California, USA, and West Nile Virus IgM Capture EIA, PANBIO, Windsor, Australia) according to the manufacturers' instructions, as

described previously.⁴ Samples positive in both IgM assays were retested using a second-generation IgM assay incorporating a background subtraction step to remove non-specific reactivity (PANBIO). Follow-up sera were requested from all patients and screened with a WNV IgG assay (West Nile Virus IgG EIA, Focus Technologies), in addition to repeat testing for WNV IgM. When the convalescent IgG test was positive, acute and convalescent sera were retested using the haemagglutination inhibition (HI) assay and confirmed by the plaque reduction neutralisation test (PRNT; antigen for HI provided by and PRNT performed at the National Microbiology Laboratory, Winnipeg, Canada).

NAAT of plasma samples for WNV RNA

Nucleic acid was extracted from 1 ml of plasma using a commercial kit (NucliSens Extraction Kit, bioMérieux, Durham, North Carolina, USA). Internal controls were spiked into each sample to monitor for extraction efficiency and inhibitory effects. Amplification of viral RNA using nucleic acid sequence-based amplification has been described previously,⁵ with modifications to the sample preparation⁶ to enable a larger volume of plasma to be analysed. Samples giving a positive result were retested using a commercially available reverse transcriptase-polymerase chain reaction assay (Artus RealArt WNV LC Assay, Qiagen Diagnostics, San Francisco, California, USA) using the LightCycler (Roche Diagnostics, Quebec, Canada) to confirm WNV infection.

Case definition and statistical analyses

As no test was likely to serve as a reliable gold standard when used alone, a case definition based on multiple test results was applied for analysis of the results. Patients were considered to be WNV cases if (1) PRNT was positive; or (2) both WNV nucleic acid sequence based amplification and reverse transcriptase-polymerase chain reaction tests were positive; (3) the WNV IgM test was positive by both commercial kits, and was subsequently positive by the second-generation PANBIO kit incorporating a background subtraction step; or (4) the WNV HI test showed a fourfold rise in titre in the absence of recent travel. Alberta is non-endemic for other flaviviruses that could cause cross-reactivity in the HI test. As 2003 was the first year of WNV activity in Alberta, long-term persistence of IgM to the virus did not complicate interpretation.⁷

All residents of Alberta who met the laboratory definition of WNV infection mentioned above were designated as "cases". All individuals tested for WNV infection by serology, NAAT or both who either tested negative on the screening assays or did not meet the laboratory case definition were designated as "non-cases". We identified significant differences in features between WNV cases and non-cases using the χ^2 test, and where

Abbreviations: EIA, enzyme immunoassay; HI, haemagglutination inhibition; NAAT, nucleic acid amplification testing; PRNT, plaque reduction neutralisation test; ProvLab, Provincial Laboratory for Public Health; WNNS, West Nile neurological syndromes; WNV, West Nile virus

Table 1 Demographic and clinical characteristics of individuals infected with West Nile virus (cases) compared with non-cases

	Sample size	WNV cases, n (%)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)*
Sex†				
Female	1371	137 (10.0)	NS (p=0.184)	
Male	1182	140 (12.0)	NS	
Age (years)‡				
0–9	156	0 (0)	ND	
10–19	276	11 (4.7)	0.3 (0.21 to 0.65) p=0.001	0.47 (0.24 to 0.88) p=0.02
20–29	329	23 (7.3)	0.6 (0.39 to 0.92) p=0.01	NS (p=0.388)
30–39	497	62 (12.5)	NS (p=0.283)	
40–49	572	84 (15)	1.6 (1.2 to 2.1) p=0.001	2 (1.4 to 2.8) p<0.001
50–59	380	56 (15.3)	1.6 (1.1 to 2.1) p=0.006	1.9 (1.3 to 2.8) p=0.002
≥60	335	40 (12)	NS (p=0.61)	
Health Region				
1 (South)	220	37 (16.8)	1.7 (1.2 to 2.5) p=0.006	2.5 (1.6 to 4) p<0.001
2 (Southeast)	367	132 (36)	7.5 (5.7 to 9.8) p<0.001	4.7 (3.4 to 6.6) p<0.001
3 (West, Calgary area)	686	42 (6.1)	0.42 (0.3 to 0.6) p<0.001	NS (p=0.1)
4 (South central)	278	30 (10.8)	NS (p=0.852)	
5 (East)	146	15 (10.3)	NS (p=0.737)	
6 (Central, Edmonton area)	713	26 (3.6)	0.23 (0.15 to 0.35) p<0.001	NS (p=0.1)
7, 8, 9 (North)	142	3 (2.1)	ND	
Clinical symptoms§				
Fever	1565	232 (14.8)	1.7 (1.2 to 2.3) p=0.002	NS (0.101)
Maculopapular rash	459	180 (39.2)	9.8 (7.4 to 13) p<0.001	8.3 (6.1 to 11.2) p<0.001
Headache	1465	215 (14.7)	1.4 (21.1 to 2) p<0.001	NS (p=0.546)
Neck rigidity	652	113 (17.3)	1.6 (1.2 to 2.1) p=0.001	NS (p=0.482)
Altered mental state	204	31 (15.1)	NS (p=0.442)	
Muscle weakness	734	140 (19.1)	2 (1.6 to 2.6) p<0.001	NS (p=0.373)
Flaccid paralysis	22	4 (18.2)	ND	
Abnormal reflexes	35	6 (17.1)	NS (p=0.521)	
Cranial nerve palsy	21	1 (4.8)	ND	
Peripheral neuropathy	71	6 (8.5)	NS (p=0.266)	
Tremor	91	28 (30.7)	3.1 (1.9 to 4.9) p<0.001	3.6 (2.1 to 6.2) p<0.001
Parkinsonism	7	1 (14.3)	ND	
SIADH	12	3 (25)	ND	
Sensory deficits	69	13 (18.8)	NS (p=0.186)	
Guillain-Barre syndrome	6	1 (16.7)	ND	
Focal lesion on imaging	15	2 (13.3)	ND	

ND, not done; NS, not significant; SIADH, syndrome of inappropriate anti-diuretic hormone; WNV, West Nile virus.

*OR was adjusted for all variables that were significant at the p<0.05 level by univariate analysis.

†Gender was unknown for five individuals, none of whom were positive for WNV infection.

‡Age was unknown for eight individuals, one of whom was positive for WNV infection.

§Clinical data forms were not received at the Provincial Laboratory for 490 individuals (16%), 5 of whom were positive for WNV infection.

appropriate, Fischer's exact test. The independent variables examined included age, gender, health region of residence and clinical symptoms. Logistical regression analyses were conducted to define independent predictors of the WNV cases. Only those results that were not affected by missing values are reported here. We did not conduct any statistical analyses for variables with a value of less than five in any given cell.

RESULTS

During the 2003 West Nile season, 2554 individuals were tested for WNV infection at ProvLab. According to the case definition given above, 277 (10.8%) individuals tested in Alberta during 2003 had laboratory evidence of WNV infection. Of these, using Alberta WNV case definitions (<http://www.health.gov.ab.ca/public/WNV-professionals.html>), 229 (82.7%) patients had West Nile fever, and 46 (16.6%) patients had West Nile neurological syndromes (WNNS). Two (0.7%) asymptomatic patients were identified during blood donor screening, and referred to ProvLab for confirmation. Clinical history sheets were received for 84% of patients. Demographic features were not significantly different in those patients who did not submit history sheets.

Table 1 shows the characteristics of WNV cases compared with non-cases. After adjusting for all other variables, age between 40–59 years, residence in Health Regions 1 (South) or

2 (Southeast) and having maculopapular rash or tremor were significantly and independently associated with WNV infection. Maculopapular rash was also associated with NAAT-documented viraemia (odds ratio 9.2, 95% CI 5.6 to 15, p<0.001).

DISCUSSION

We were able to conduct a population-based analysis of clinical factors associated with WNV infection by comparing WNV cases with a control group of symptomatic patients in whom no WNV infection was documented. Maculopapular rash, present in 63.4% of cases, proved to be the symptom with the highest predictive value for WNV infection. This may reflect the greater proportion of early cases identified using NAAT, as maculopapular rash was also associated with plasma viraemia. In addition, our patient population, encompassing all patients tested for WNV in the province, covered a wide range of the disease spectrum, and was not limited to severe neurological cases. Most previous studies reporting infrequent rash are limited to WNNS cases,^{1–3} whereas most of our cases were classified as West Nile fever. Of our WNNS cases, only 37% had a rash, which is closer to the previous reports (16–27%). Thus, maculopapular rash could serve as an early clinical clue to the onset of WNV activity in the community.

In previous reports, small case series, muscle weakness, flaccid paralysis and meningitis/encephalitis/meningoencephalitis have

often been identified as characteristic neurological features of WNV infection. Tremor and transient parkinsonism have also been described.⁸ Such movement disorders are consistent with neuropathology and imaging studies of WNV and related flaviviruses that show inflammation of the basal ganglia and substantia nigra.^{9–12} In this report, of the neurological features studied, tremor was the most predictive for WNV infection. This suggests that involvement of the basal ganglia may be more characteristic for WNV infection than recognised previously, and that WNV disease syndromes historically classified as WNV neurological syndromes may represent only the more severe end of the neurological disease spectrum. A limitation of this study is that neurological examination was not performed in a standardised manner. The statistically significant association with tremor, as understood by the doctor, warrants more rigorous clinical evaluation.

In summary, using combined molecular and serological diagnosis on a population basis, we observe that age, location, maculopapular rash and tremor are the features most predictive of acute WNV infection.

ACKNOWLEDGEMENTS

We thank the technologists at ProvLab for assay set-up and diagnostic testing, particularly Vinod Khurana and Roberta Walle. Dr R Lanciotti (CDC, Fort Collins) and Dr M Drebot (NML, Winnipeg) provided assistance for molecular and serological assay set-up. West Nile virus diagnostic testing was supported by Alberta Health and Wellness.

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Competing interests: None declared.

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Received 27 September 2006

Revised 28 November 2006

Accepted 30 November 2006

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