

No evidence of West Nile virus infection among Polish patients with encephalitis

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Abstract

West Nile virus (WNV) infection usually causes mild febrile illness, but in a small proportion of patients it can lead to encephalitis. Epidemiological studies of WNV indicate fast spread of infection worldwide and in Europe, but there have been no comprehensive studies of WNV infection among encephalitis patients in Poland.

Here we present the results of WNV RNA and anti-WNV testing in serum and cerebrospinal fluid (CSF) samples in 80 patients with the clinical diagnosis of viral encephalitis. WNV RNA was not detected in any of the analyzed samples. Anti-WNV IgG and IgM were not present in CSF in any of the investigated patients, but anti-WNV IgM were unexpectedly detected in serum of 14 subjects. The latter represented false positive results are probably related to cross reactivity of antibodies. Although there was no evidence of WNV infection in any of our patients, epidemiological situation in the neighbouring countries warrants vigilance and appropriate measures, including introduction of specific diagnostic tools into clinical practice, seem necessary.

Key words: West Nile virus, neuroinfection, emerging infection, Flaviviridae.

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Introduction

West Nile virus (WNV) was first described in 1937 in Uganda as an etiological agent of mild febrile illness [1]. Although most WNV infections in humans are asymptomatic or present with benign flu-like syndrome only, up to 1% of infected subjects progress to more severe disease [2-4]. Clinically overt infection is more frequent among elderly or immunocompromised patients and in subjects with co-existing chronic medical conditions like diabetes or renal failure [5, 6]. WNV can be responsible for a considerable proportion of encephalitis and meningitis cases, 3-19% of which have fatal outcome [1, 7, 8].

Epidemiological studies of WNV indicate fast and extensive spread and there are well documented cases of WNV infection in Romania (Bucharest 1996), Russia (Volgograd 1999), USA (New York City 1999), and Israel (2000) [9-11]. After the initial appearance of WNV in New York, WNV-related encephalitis cases were reported in different areas of the USA, as well as in Mexico, and Canada, Central and South America [12, 13], and WNV is currently recognized as one of the most important causes of viral encephalitis worldwide [1].

Encephalitis is the most common form of WNV neuroinvasion (50-71%), followed by meningitis (15-35%) and acute flaccid paralysis [14, 15]. However, Guillain-Barré syndrome and brachial plexus palsy were also reported to be associated with this virus [16-18]. Of note, a significant proportion of patients recovering from the infection develop mental and physical sequelae [19]. Similarly to other Flaviviridae family members, WNV infection may persist for many years, although its consequences remain currently unclear [20].

Until now there have been no comprehensive studies of WNV infection among encephalitis patients in Poland as previous reports analyzed either very small groups of patients with meningitis or employed serodiagnostic screening only [21, 22]. Here we present the results of WNV-RNA and anti-WNV testing in serum and cerebrospinal fluid samples (CSF) in a large group of patients with clinical diagnosis of viral encephalitis.

Material and methods

Patients

Eighty patients (42 men, 38 women) diagnosed as having encephalitis and hospitalized in recent years in the

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Clinic of Infectious Diseases for Adults, Warsaw Medical University, were included in the study. The most common complaints were headache (51.3%) and fever (43.8%). At admission 20% of patients had decreased consciousness, 18.8% had meningeal signs and 20% had focal neurological signs. In only one case the course of encephalitis was fatal.

Anti-WNV IgM and IgG analysis

CSF and serum samples for all patients were tested for anti-WNV IgM and IgG class by commercially available kits (anti-West Nile Virus IgM & IgG ELISA; Euroimmun, Lubeck, Germany) following procedure recommended by the manufacturer.

WNV RNA detection

Total RNA was extracted from serum and CSF samples by modified Chomczynski method employing Trizol reagent (Invitrogen, Carlsbad, USA). The obtained RNA was subjected to reverse transcription with random hexamers (Invitrogen, Carlsbad, USA) followed by amplification of viral 5'UTR region with primers (forward) 5'-AGTAGTTCGCCTGTGTGAGC-3' and (reverse) 5'-GCCCTCCTGGTTTCTTAGA-3'. The PCR run was performed using LightCycler FastStart DNA Master SYBR Green I (Roche Diagnostics, Switzerland) employing LightCycler 2.0 (Roche Diagnostics, Switzerland). Each of the 50 cycles consisted of 95°C (30 s), 57°C (5 s), and 72°C (30 s), and fluorescence measurement was at 85°C (1 s).

Table 1. Clinical and laboratory characteristics of patients with encephalitis ($n = 80$)

Characteristics	
Age (years; mean \pm SD)	42.78 \pm 16.12
Gender	
Male, n (%)	42 (52.5%)
Female, n (%)	38 (47.5%)
Major complaints	
Fever, n (%)	35 (43.8)
Headache, n (%)	41 (51.3)
At admission	
Decreased consciousness, n (%)	16 (20.0)
Seizures, n (%)	13 (16.3)
Meningeal signs, n (%)	15 (18.8)
Focal neurological signs, n (%)	16 (20.0)
CSF analysis	
Cytosis (in 1 μ l), mean \pm SD	82.34 \pm 172.14
Proteins (g/l), mean \pm SD	0.74 \pm 0.56
Glucose (mmol/l), mean \pm SD	3.68 \pm 1.12

The positive control samples consisted of commercially available WNV AmpliRun West Nile Virus RNA (Vircell S.L., Grenada, Spain) containing 12.500-20.000 WNV copies/1 ml. Using dilutions of the above standard we found that it was able to detect 8-13 viral copies per reaction.

Results

The results of clinical and laboratory analysis are presented in Table 1.

Neither WNV RNA nor anti-WNV IgG were detected in any of the CSF or serum samples. However, while anti-WNV IgM were not present in CSF they were detected in serum in 14 subjects.

Discussion

Viral neuroinfections transmitted by vectors represent a rising health problem worldwide. In Poland, the dominant form of vector-transmitted neuroinfection is tick borne encephalitis virus (TBEV), which was long confined to defined endemic regions, but now is seen throughout the country [23].

The presence of WNV infection was reported in such European countries as Greece, France, Romania, Hungary, Italy, Portugal and Spain, as well as in Poland's direct neighbours: the Czech Republic, Slovakia, Belarus and Ukraine [1]. Moreover, 12 out of 46 vectors capable of transmitting WNV (most prominently *Culex pipiens*) are common in Central and Eastern Europe and are also present in Poland [24]. Climate warming extends the habitat range of transmitting mosquitos as well as the duration of their seasonal activity [25].

West Nile Virus infection clearly represents a threat to Poland as it is already well-established in Europe including Poland's immediate neighbours. In support of the presence of WNV in Poland come reports on the detection of anti-WNV-specific IgM in a patient suffering from fever of unknown etiology [24] and the detection of anti-WNV in a significant proportion of wild birds as well as in horses [26, 27].

Surprisingly, WNV RNA was not detected in any of our 80 encephalitis patients neither in serum nor in CSF. At the same time, 14 patients were anti-WNV IgM positive in serum. These findings are not necessarily discrepant, because the diagnostic relevance of serological screening for WNV infection is severely limited by the common antigenic cross-reactivity, which often leads to false positive results [28]. Thus, the immune response directed against viral envelope (E) protein can also be reactive against more conserved among large Flaviviridae family membrane and non-structural proteins [29].

Further support for the lack of WNV infection in Poland comes from a large study of wild birds, in which no

WNV RNA was found in any of over 2000 birds tested [27]. Nevertheless, this epidemiological situation may rapidly change because WNV is well established in Europe and the vectors capable of transmitting infection are locally present.

In conclusion, WNV RNA was not found in any of the 80 encephalitis patients studied. However, the epidemiological situation in the neighbouring countries warrants vigilance and appropriate measures, including introduction of specific diagnostic tools into clinical practice, as well as constant monitoring of birds and mosquitoes, seem prudent.

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