

Human West Nile virus infection in Bosnia and Herzegovina

Sead Ahmetagić¹, Jovan Petković¹, Mirsada Hukić², Arnela Smriko-Nuhanović¹, Dilista Piljić¹

¹Clinic for Infectious Diseases, University Clinical Centre Tuzla, Tuzla, ²International Burch University, Sarajevo; Bosnia and Herzegovina

ABSTRACT

Aim To describe the first two cases of West Nile virus (WNV) neuroinvasive infections in Bosnia and Herzegovina.-

Methods At the Clinic for Infectious Diseases of the University Clinical Centre Tuzla, Bosnia and Herzegovina (B&H), specific screening for WNV infection was performed on patients with neuroinvasive diseases from 1 August to 31 October 2013. Serum samples were tested for the presence of WNV IgM and IgG antibodies using enzyme-linked immunosorbent assay (ELISA); positive serum samples were further analyzed by detection of WNV nucleic acid of two distinct lineages (lineage 1 and lineage 2) in sera by RT-PCR.

Results Three (out of nine) patients met clinical criteria, and two of them had high serum titre of WNV specific IgM antibodies (3.5 and 5.2). Serum RT-PCR testing was negative. Confirmation by neutralization testing was not performed. Both cases represented with encephalitis. None of these cases had recent travel history in WNV endemic areas, or history of blood transfusion and organ transplantation, so they represented autochthonous cases.

Conclusion Although there were no previous reports of flavivirus infections in B&H, described cases had high titre of WNV specific antibodies in serum, and negative flavivirus-vaccination history, they were defined as probable cases because recommended testing for case confirmation was not performed. The West Nile virus should be considered a possible causative pathogen in this area, probably in patients with mild influenza-like disease of unknown origin and those with neuroinvasive disease during late summer and early autumn.

Key words: neuroinvasive infections, encephalitis, flaviviruses

Corresponding author:

Arnela Smriko-Nuhanović
Clinic for Infectious Diseases,
University Clinical Centre Tuzla
Trnovac bb, 75 000 Tuzla,
Bosnia and Herzegovina
Phone: +387 35 303 326;
Fax: +387 35 303 480;
E-mail: asmriko@hotmail.com

Original submission:

14 August 2014;

Revised submission:

24 October 2014;

Accepted:

18 December 2014.

INTRODUCTION

The genus *Flavivirus* comprises 53 viruses, and many of them are human pathogens of concern. In Europe, many flaviviruses are endemic (West Nile, Usutu, tick-borne encephalitis viruses) or occasionally imported (dengue, yellow fever viruses) (1). West Nile virus (WNV) is transmitted in an avian cycle by ornithophilic mosquitoes, chiefly of the genus *Culex*. Mammals can also be infected, but are considered dead end hosts because viraemia is generally too low to infect mosquitoes (2). West Nile virus was first identified in the West Nile district of Uganda in 1937 in a woman who presented with a mild febrile illness (3). During the next decades, the virus spread through Africa and Asia. It was first described in Europe in the 1960s when seropositive animals or virus isolates were reported in France, Portugal and Cyprus (4). West Nile virus has historically been considered less pathogenic in humans than dengue virus or yellow fever virus; however, more virulent genotypes have emerged since 1998. Isolates from Israel (1998), Hungary (2003), or North America (1999) belonging to the Israeli-American cluster of WNV lineage 1a are highly pathogenic in birds and mammals, and lineage 2 viruses have caused an increasing number of WNV outbreaks in Europe since 2008 (2). In Europe, WNV has mainly been reported in central and south-eastern Europe, regions in which WNV infections and virulence have recently increased, and the implicated viruses have spread to new areas, including Bulgaria and Greece in 2010, Albania and Macedonia in 2011, and Croatia, Serbia, and Kosovo in 2012 (1,2,5). The first laboratory-confirmed cases of the West Nile virus neuroinvasive infection in Croatia were diagnosed in September 2012 in three eastern Croatian counties, although specific antibodies to West Nile virus in humans, horses, and European brown bears have been previously detected (6-9). Also, in Serbia first outbreak of West Nile virus infection was reported in humans in August to October 2012, and evidence of detected virus activity in horses, wild birds and mosquitos (10-12). There are no reports of WNV or other flaviviruses activities in humans or animals in Bosnia and Herzegovina.

PATIENTS AND METHODS

At the Clinic for Infectious Diseases University Clinical Centre Tuzla, Bosnia and Herzegovina

(B&H), specific screening for WNV infection was performed on patients with neuroinvasive diseases with negative CSF bacterial cultures and negative serological tests for other common causes of bacterial, viral or protozoan meningitis and/or encephalitis (rubella, measles, mumps, varicella-zoster viruses, adenovirus type 3, Epstein-Barr, herpes simplex virus-1, herpes simplex virus-2, cytomegalovirus, coxsackie B, coxsackie A7, echovirus type 7 viruses, *Treponema pallidum*, *Borrelia burgdorferi* sensu stricto, *Borrelia garinii*, *Borrelia afzelii*, *Haemophilus influenzae*, *Listeria monocytogenes* 1/2a and 4b, *Toxoplasma gondii*) from 1 August to 31 October 2013. There was no data about specific WNV screening performed before in medical centres in B&H. The study had been approved by the Research Ethics Committee of the University Clinical Centre Tuzla.

Case definition

The case definition and case classification were established according to European Union case definitions for West Nile fever (13). Persons with fever (≥ 37.5 °C) and meningitis and/or encephalitis were included. Meningitis was defined as presence of fever, clinical signs of meningeal inflammation (including headache, nuchal rigidity, Kernig's sign or Brudzinski signs, photophobia or phonophobia), and the presence of cerebrospinal (CSF) pleocytosis (>5 leucocytes/mm³), elevated protein levels (>0.45 g/L; normal range 0.15-0.45 g/L) and normal (2.6-3.1 mmol/L, 50-60% serum glucose levels) or mildly decreased (2.4-2.6 mmol/L) CSF glucose level. Encephalitis was defined as the presence of fever, encephalopathy (decreased or altered level of consciousness, lethargy or personality change) and/or focal neurological signs (weakness, cranial nerve palsy), seizures or movement disorders (tremor, parkinsonism, ataxia), and the presence of CSF pleocytosis (>5 leucocytes/mm³), elevated protein levels (>0.45 g/L), and normal (2.6-3.1 mmol/L, 50-60% serum glucose levels) or mildly decreased (2.4-2.6 mmol/L) CSF glucose level.

Laboratory criteria for a probable case were the presence of WNV-specific antibody response in serum (IgM or IgM and IgG). Laboratory criteria for a case conformation were isolation of WNV from blood or CSF, detection of WNV nucleic acid in blood or CSF, WNV specific antibody

response (IgM) in CSF, WNV IgM high titre and detection of WNV IgG, and conformation by neutralisation.

Serology and molecular tests

Serum samples were tested at the Institute of Clinical Microbiology, University Clinical Center of Sarajevo, for the presence of WNV IgM and IgG antibodies using the West Nile Virus IgM Capture DxSelect™ ELISA and West Nile Virus IgG DxSelect™ ELISA kits (Focus Diagnostics, Cypress, California, USA). Serum samples, which tested positive in the ELISA, were further analyzed by detection of WNV nucleic acid of two distinct lineages (lineage 1 and lineage 2) in sera by RT-PCR. Results were confirmed at the Institute of Microbiology and Immunology, Medical Faculty University of Ljubljana, Slovenia. Diagnostic tests for WNV isolation from blood, specific CSF testing (WNV isolation, specific antibody response, or detection of nucleic acid), and conformation by neutralization were not performed.

Patient data

Patient's demographic characteristics (age, sex, region of residence), travel history, blood transfusions, transplants, and vaccination status, comorbidity, presenting symptoms and signs, clinical findings, hematology and blood biochemistry analysis, CSF cell count and biochemical analysis were recorded, along with serology and molecular diagnostic tests results, as well as the outcome at hospital discharge.

RESULTS

During the period 01 August to 31 October 2013 only three patients (out of nine) met clinical criteria, and two of them were identified as probable cases according to the case definition (Table 1); conformation by neutralization testing was not performed.

Table 1. Results of serology and molecular testing of two patients with neuroinvasive West Nile virus infection

Case	Sample type	Day of sampling after illness onset	IgM ELISA (titre)	IgG ELISA (titre)	RT-PCR
1	Serum	20	Positive (3.5)	Positive (2.2)	Negative
2	Serum	12	Positive (5.2)	Negative (0.27)	Negative

First case had onset of symptoms at the beginning of August, and second case at the beginning of September 2013. None of these cases had recent travel history in areas where WNV was endemic, nor the history of blood transfusion and organ transplantation, and had negative flavivirus-vaccination history (demographic characteristics and comorbidities are shown in Table 2). They were both represented with encephalitis (self-reported symptoms and clinical signs are shown in Table 3).

Table 2. Demographic characteristics and comorbidities of two patients with neuroinvasive West Nile virus infection

Case	Age (years)	Sex	City area	Immunosuppression including diabetes	Hypertension	Other chronic illness
1	84	Male	Tuzla	No	Yes	Vertigo Chronic bronchitis
2	68	Female	Kladanj	No	Yes	Hypertensive heart disease

Table 3. Self-reported symptoms and clinical signs of two patients with neuroinvasive West Nile virus infection

Signs and symptoms	Case 1	Case 2
Fever ≥ 37.5 °C	+	+
Neurological manifestation	+	+
Fatigue	+	+
Consciousness impairment	+	+
Rash	+	-

+, present; -, absent;

Neurological manifestation, including neurological deficit and consciousness impairment were recorded on day 10 after symptom onset in both cases.

Neurological manifestations in case 1 included horizontal nystagmus, positive Romberg's sign, positive Kernig's sign, neck and extremities muscle stiffness with lively deep tendon reflexes, and urine retention. In this case qualitative (confusion, disorientation) and quantitative (somnolence) consciousness impairment were present, and mood changes in form of anxiety.

Neurological manifestations in case 2 included ataxia, horizontal nystagmus, positive Romberg's sign, nuchal rigidity, and diminished deep tendon reflexes, with qualitative (confusion, disorientation) and quantitative (somnolence) consciousness impairment, and incoherent speech.

Cerebrospinal fluid analysis results of these two cases are shown in Table 4.

None of these patients developed respiratory failure requiring mechanical ventilation. Both patients recovered by the time they were discharged, case 2 had complete recovery, and case 1 still had

mild horizontal nystagmus, positive Romberg's sign and urine retention.

Table 4. Cerebrospinal fluid analyses results of 2 patients with neuroinvasive West Nile virus infection

Parameter	Normal value/range	Case 1	Case 2
WBC (per mm ³)	<5	136	960
WBC differential	No predominance	60% L	62% N
Protein (g/L)	<0.45	1.55	1.45
Glucose (mmol/L)	2.6-3.1 mmol/L	3.3	2.7

WBC, white blood cells; L, lymphocytes; N, neutrophiles;

DISCUSSION

Taking in consideration that large WNV outbreaks happened in Eastern Croatia (6) and Serbia (10) in 2012, we analyzed appearance of the human neuroinvasive disease in B&H, performing a screening for patients hospitalized throughout summer and autumn 2013 for WNV infection with the aim to investigate presence of the virus in this location. Although only patients with neuroinvasive disease were tested, of whom two were positive, we assumed that there were more patients with WNV infection who had influenza-like febrile illness who were not tested, or who did not seek medical help due to the symptoms being too mild or were treated in primary care. This is a single-center study at regional level, and a large study at state level should be planned so that the incidence rates can be calculated.

Both presented patients had clinical signs of encephalitis that could not be distinguished from the patients with encephalitis with a different etiology (14), but large sample case-control studies might reveal specific characteristic of encephalitis due to WNV infection (14).

Serological diagnosis of flavivirus infections is complicated by the antigenic similarities among the Flavivirus genus because most flavivirus antibodies are directed against the highly immunogenic envelope protein, which contains both flavivirus cross-reactive and virus-specific epitope (1). Serological assay results should thus be interpreted with care and confirmed by comparative neutralization test using a panel of viruses known to

REFERENCES

1. Beck C, Jimenez-Clavero MA, Leblond A, Durand B, Nowotny N, Leparc-Goffart I, Zientara S, Jourdain E, Lecollinet S. Flavivirus in Europe: Complex circulation patterns and their consequences for the diagnosis and control of West Nile Disease. *Int J Environ Res Public Health* 2013;10:6049-83.

circulate in Europe (1,15). Although there have been no previous reports of other flavivirus infections in B&H, the cases presented in this report had high titre of WNV specific antibodies in serum, and had negative flavivirus-vaccination history, and accordingly, they were defined as probable because we were not able to perform recommended testing for case confirmation.

Knowledge of flavivirus diversity at the local level is essential before rigorous serological surveys can be undertaken (1). Veterinary and entomologic investigations related to WNV and other flaviviruses have not been performed in B&H, so we can only assume from the published regional data (6-12) the circulating pattern of the virus in this area. Systematic research is needed to understand epidemiology and ecology of WNV in B&H. It is very important to develop a project to detect WNV in mosquitoes in urban regions and near rivers in various parts of the state, as well as surveillance of human cases.

Considering the vectors and recent floods, a number of public health measures can be undertaken (intensifying activities to reduce the number of mosquitos in environments using insecticide, destroying mosquito habitat, systematic extermination of larvae and adult form of larvae and adult form of mosquitoes, education of the population on how to avoid or decrease the risk of being bitten by potentially infected mosquitoes through posters, leaflets, television, and newspapers) (16). These actions should help avoid outbreaks of human WNV infection and raise awareness of healthcare providers about emergence of the virus in B&H. In patients with mild influenza-like disease of unknown origin and those with neuroinvasive disease during late summer and early autumn, WNV should be considered a possible causative pathogen.

FUNDING

No specific funding was received for this study.

TRANSPARENCY DECLARATION

Competing interests: None to declare.

2. Reiter P. West Nile virus in Europe: understanding the present to gauge the future. *Eurosurveill* 2010. <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19508>. (3 Jun 2014)
3. Sithburn KC, Hughes TP, Burke AW Paul JH. A neurotropic virus isolated from the blood of a native of Uganda. *Am J Tro Med* 1940; 20:471-92.

4. Murgue B, Murri S, Triki H, Deubel V, Zeller H. West Nile in Mediterranean basin:1950-2000. *Ann N Y Acad Sci* 2001; 951:117-26.
5. Nowotny N, Bakonyi T, Weissenböck H, Seidel B, Kolodziejek J, Sekulin K, Lussy H. West Nile virus infection in Europe – general features. *Medica Sciences* 2013; 39:123-4.
6. Merdić E, Perić L, Pandak N, Kurolt IC, Turić N, Vignjević G, Stolfi I, Milas J, Bogojević MS, Markotić A. West Nile virus outbreak in humans in Croatia, 2012. *Coll Antropol* 2013; 37:943-7.
7. Madić J, Huber D, Lugović B. Serologic survey for selected viral and rickettsial agents of brown bears (*Ursus arctos*) in Croatia. *J Wildl Dis* 1993; 29:572-6.
8. Madić J, Savini G, Di Gennaro A, Monaco F, Jukić B, Kovac S, Rudan N, Listes E. Serological evidence for West Nile virus infection in horses in Croatia. *Vet Rec* 2007; 160:772-3.
9. Barbić L, Listeš E, Katić S, Stevanović V, Madić J, Starešina V, Labrović A, Di Gennaro A, Savini G. Spreading of West Nile virus infection in Croatia. *Vet Microbiol* 2012; 159:504-8.
10. Popović N, Milošević B, Urošević A, Poluga J, Lavadinović L, Nedeljković J, Jevtović D, Dulović O. Outbreak of West Nile virus infection among humans in Serbia, August to October 2012. *Euro Surveill* 2013. <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20613> (11 July 2014)
11. Petrović T, Blázquez AB, Lupulović D, Lazić G, Escobedo-Romero E, Fabijan D, Kapetanov M, Lazić S, Saiz J. Monitoring West Nile virus (WNV) infection in wild birds in Serbia during 2012: first isolation and characterisation of WNV strains from Serbia. *Euro Surveill* 2013; 31:18. pii: 20622.
12. Lupulović D, Martín-Acebes MA, Lazić S, Alonso-Padilla J, Blázquez AB, Escobedo-Romero E, Petrović T, Saiz JC. First serological evidence of West Nile virus activity in horses in Serbia. *Vector Borne Zoonotic Dis* 2011; 11:1303-5.
13. European Center for Disease Prevention and Control (ECDC). EU case definition. Stockholm: ECDC. http://ecdc.europa.eu/en/healthtopics/west_nile_fever/EU-case-definition/Pages/EU-case-definition.aspx (10 October 2013)
14. Sejvar JJ. Clinical manifestations and outcomes of West Nile virus infection. *Viruses* 2014; 6:606-23.
15. Sambri V, Capobianchi MR, Cavrini F, Charrel R, Donoso-Mantke O, Escadafal C, Franco L, Gaibani P, Gould EA, Niedrig M, Papa A, Pierro A, Rossini G, Sanchini A, Tenorio A, Varani S, Vázquez A, Vocale C, Zeller H. Diagnosis of West Nile virus human infections: overview and proposal of diagnostic protocols considering the results of external quality assessment studies. *Viruses* 2013; 5:2329-48.
16. Radivojević S, Maris S, Ljubić B, Obrenović J. First detection of West Nile fever in human population in the territory of Belgrade. *Proceedings Third International Epizootiology Days and XV Serbian Epizootiology Days, Niš/Serbia, May 8-11 2013. Faculty of veterinary medicine Belgrade, University of Belgrade, Department for infectious disease of animals and bee diseases, Belgrade. FVM; 2013. p. 61-2.* http://www.vet.bg.ac.rs/uploads/file/seminari_simpozijumi/Epizootiološki.dani.2013.ZBORNIK.pdf (20 April 2014)

Humana infekcija virusom Zapadnog Nila u Bosni i Hercegovini

Sead Ahmetagić¹, Jovan Petković¹, Mirsada Hukić², Arneta Smriko-Nuhanović¹, Dilista Piljić¹

¹Univerzitetski klinički centar Tuzla, Tuzla, ²International Burch University, Sarajevo; Bosna i Hercegovina

SAŽETAK

Cilj Prikazati prva dva slučaja neuroinvazivne infekcije uzrokovane virusom Zapadnog Nila (WNV) u Bosni i Hercegovini (BiH).

Metode U Klinici za infektivne bolesti Univerzitetskog kliničkog centra Tuzla (BiH), u periodu od 1. avgusta do 31. oktobra 2013. godine, kod pacijenata kojima je postavljena dijagnoza neuroinvazivne infekcije provedena su specifična testiranja na WNV infekciju. Uzorci seruma testirani su na prisustvo specifičnih WNV IgM i IgG antitijela pomoću enzimsko-imunološkog testa (ELISA), a pozitivni uzorci seruma su dalje testirani ispitivanjem prisustva WNV nukleinske kiseline za dvije različite linije (linija 1 i linija 2) pomoću RT-PCR-a.

Rezultati Samo tri pacijenta (od ukupno devet) zadovoljavala su postavljene kliničke kriterije, od kojih su dva imala visoke titrove WNV specifičnih IgM antitijela (3,5 i 5,2) u serumu. RT-PCR testovi iz istog seruma bili su negativni. Potvrđni test neutralizacije nije urađen. Oba slučaja imala su kliničku sliku encefalitisa. Pacijenti nisu boravili u područjima gdje je virus Zapadnog Nila endemičan, niti su primali transfuzije krvi ili transplantaciju organa, tako da predstavljaju autohtone slučajeve.

Zaključak Iako nije bilo ranije prijavljenih flavivirusnih infekcija u BiH, u oba slučaja su u serumu nađena specifična WNV antitijela u visokom titru i u oba slučaja nije bilo prethodne vakcinacije protiv flavivirusa; oba slučaja su ostala nepotvrđena jer nije provedeno preporučeno testiranje za potvrdu slučaja. Virus Zapadnog Nila je prisutan u ovoj regiji te ga uvijek treba razmotriti kao potencijalnog patogena u pacijenata sa simptomima blage influence nepoznatog porijekla ili neuroinvazivne bolesti koji se javljaju u kasno ljeto i ranu jesen.

Ključne riječi: neuroinvazivne infekcije, encefalitis, flavivirusi