CASE REPORT ON TWO CONSECUTIVE PATIENTS WITH NEUROINVASIVE WEST NILE VIRUS INFECTION IN AN INTENSIVE CARE UNIT

Silviya Stoyanova[1]*, Raynichka Mihaylova-Garnizova[2], Emilia Naseva[3,4], Severina Dakova[1], Iva Christova[5], Konstantin Ramshev[1]

[1] Clinic of Intensive Care, Military Medical Academy – Sofia, Bulgaria
[2] Clinic of Infectious Diseases, Military Medical Academy – Sofia, Bulgaria
[3] Department of Health Management and Health Economics, Faculty of Public Health "Prof. Tsekomir Vodenicharov, MD, DSc", Medical University of Sofia, Bulgaria
[4] Medical Faculty, Sofia University St. Kliment Ohridski, Sofia, Bulgaria
[5] Department of Microbiology, National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria

ABSTRACT

We present the clinical course, and treatment of two consecutive cases of neuroinvasive West Nile virus (WNV) infection in the Clinic of Intensive Care, an Intensive Care Unit (ICU) at the Military Medical Academy - Sofia. Clinical and epidemiological data, microbiological, laboratory, molecular methods, and imaging techniques were used. Both patients resided in Sofia, Bulgaria, and had not travelled in recent months. The first case was a 60-year-old man who have had mental status changes, fever, and progression of existing Parkinson's disease. Antibodies to WNV were present in cerebrospinal fluid (CSF). His condition worsened with the development of sepsis and respiratory failure and he ended up lethally. The second case was a 72-year-old man who had fever and lower dyspeptic syndrome for one week, mental status changes, with adynamia to inability to walk independently, and head, hand and tongue tremors. CSF analysis showed mild pleocytosis with proteinorachy. Antibodies to WNV were present in serum, and PCR for WNV in urine was positive. The patient was admitted to ICU due to worsened mental and neurological status, coma and development of acute respiratory failure, necessitating intubation and assisted pulmonary ventilation. The patient ended lethally 13 days later. Neuroinvasive WNV infection can cause substantial morbidity, particularly among older adults, and high mortality, in the presence of comorbidities. Physicians should include West Nile virus infection in the differential diagnosis of aseptic meningitis and encephalitis, should perform appropriate laboratory tests, and report immediately the cases to the public health authorities.

Keywords: West Nile virus, fever, neuroinvasive disease, encephalitis

ADDRESS FOR CORRESPONDENCE:
Silviya Stoyanova
Clinic of Intensive Care, Military Medical Academy
1 Georgi Sofiyski Blvd, 1606, Sofia, Bulgaria
phone: +359885711232
email: dr.silvia.stoyanova@abv.bg

INTRODUCTION

West Nile virus (WNV) is an RNA virus classified as arbovirus. WNV belongs to *Flaviviridae* family including Dengue, Yellow fever, and Japanese encephalitis virus. WNV was named after the region where in 1937 was isolated for the first time in Uganda. Today, WNV is one of the arboviruses with the largest geographical spread and can be found in all continents [1]. The disease impacts countries in all parts of Europe, except for the Northern one [1, 2]. Several cases of West Nile encephalitis have been reported in Bulgaria in previous years [3-5].

WNV is transmitted through an enzootic cycle involving mosquitoes and birds, which act as vectors and amplifying hosts, respectively. Several bird species serve as effective reservoirs for infected mosquitoes. Among mosquitoes, Culex species play the key role for transmission [6]. The disease is most commonly transmitted to humans through bites from infected mosquito. Humans, as well as other mammals, are accidental hosts and do not cause significant additional spread of the virus as they do

not develop a sufficiently prolonged or high viremia [6, 7]. Human-to-human transmission is uncommon but can happen after hem transfusion, organ or tissue transplantation, and mother-to-child transmission during pregnancy or lactation [8].

Approximately 80% of human WNV infections are asymptomatic [9]. Some 20% of infected persons can have symptomatic viral infection and less than one percent can develop West Nile neuroinvasive disease [9]. In rear cases, WNV infection could cause nerve disorders involving demyelination such as Guillain-Barré syndrome [10].

The incubation period of WNV infection is 2 to 6 days and up to 21 days in immunocompromised hosts. West Nile fever (WNF) is characterized by a sudden presentation of symptoms, including headache, eye pain, fever, malaise, myalgia, fatigue, rash, vomiting, and diarrhea [10]. The symptoms may vary from a mild self-limiting health issue from which patients heal for a week, to a prolonged disorder that can become severely debilitating [11, 12].

West Nile neuroinvasive disease (WNND) presents with a critical clinical course, possible fatal outcome, and is commonly associated with neurological complications in survivors. Advanced age, malignancies, alcohol abuse, some comorbidities such as arterial hypertension, diabetes mellitus, hematologic diseases, renal disease, as well as genetic factors increase the risk of developing WNND [10, 13]. The mortality rate of WNND is up to 17% [14, 15].

Neuroinvasive disease develops when WNV crosses the blood-brain barrier and affects specific groups of neurons, especially in the deep nuclei, brainstem, and anterior horn of the spinal cord [16]. The clinical characteristics of WNND vary and may include encephalitis, aseptic meningitis, and polio-like syndrome [12]. More than a third of WNND patients develop meningitis, some 55-60% will have encephalitis, and nearly 5-10% will have polio-like syndrome; the presentation varies based on the region or the season. Patients could also develop overlap syndromes [16].

The symptoms of WNV encephalitis are close to other viral brain inflammations. A key finding in West Nile virus encephalitis is muscle weakness (reported for 30-50%), frequently accompanied with lower motor neuron symptoms, flaccid paralysis, and hyporeflexia without sensory abnormalities [12]. Other WNND symptoms include cranial nerves involvement (notably the seventh) and motor disorders.

The diagnosis of WNND is based on factors such as exposure to the vector, residence in an endemic area, summer season, and clinical symptoms. Laboratory methods for diagnosis of WNV infection are mostly indirect, based on serology as well as the direct detection of the virus. A cerebrospinal fluid (CSF) sample is needed in case of neurological sequelae. Indirect detection of WNV-specific IgM and/or IgG antibodies is mostly grounded on the principles of Enzyme-linked immunosorbent assay (ELISA) or indirect immunofluorescence assay (IIFA) [1]. WNV infection can be confirmed by detection of the viral genome by real-time polymerase chain reaction (RT-PCR) [17].

PCR testing of serum or cerebrospinal fluid have limitations for diagnosis of WNV in immunocompetent patients because the peak of viremia occurs three to four days before the onset of symptoms. The sensitivity of PCR for WNV is low in CSF (57%), and serum (14%) [18]. PCR sensitivity may be greater in immunocompromised patients with a weakened antibody development and prolonged viremia [16]. CSF testing results in WNND patients are nonspecific and could show pleocytosis (prevalence of lymphocytes or neutrophils) with increased protein and normal glucose levels [19].

Imaging tests, such as magnetic resonance imaging (MRI), are also nonspecific and the results from different series are inconsistent. In some series, MRI results showed no abnormalities, whereas in others, up to 70% of patients displayed abnormal findings [20-22].

At present, there is no specific treatment for WNV infection and disease management relies on supportive measures. Analysis of the literature reveals the availability of several effective antiviral drugs against the WNV pathogen [23]. Thus, experts have demonstrated the activity of ribavirin and interferon alfa-2b *in vivo* [24] and *in vitro* [25,26]. High doses of ribavirin were shown to inhibit WNV replication and cytopathogenicity in human nerve cells *in vitro* [25]. Israeli researchers have constructed a homologous immunoglobulin [27] providing effective treatment

for severe cases of WNV.

Remdesivir can effectively inhibit RNA-dependent RNA polymerases from viruses that cause infections such as Zika, West Nile fever, Yellow fever, Japanese and tick-borne encephalitis, and Dengue. Thus, Remdesivir or its derivatives have the potential of a broad-spectrum treatment effective against various RNA viruses [28, 29].

All reviewed guidelines for encephalitis treatment recommend the empiric initiation of antibiotics and acyclovir as soon as possible while awaiting diagnostic test results [30].

Case 1

A 60-year-old man was admitted to the ICU with complaints of high fever of up to 40°C lasting 3-4 days, severe weakness, low level of urine (oligouria) and shortness of breath. According to his relatives, he had been bedridden for the past few days, with restricted movements of the whole body, stiffness, confusion, and difficult speaking. He had a history of Parkinson's disease and arterial hypertension.

The initial examination found a male in severely impaired general condition, in contact, completely inadequate, and disoriented. He was febrile (39°C) and bedridden. A moderately marked tremor, perioral cyanosis, and reduced subcutaneous adipose tissue were observed. The patient had prolonged expiration, crepitations at the lungs' bases, and single dry wheezings. Oxygen saturation was 81% on ambient air. Cardiovascular system examination showed a rhythmic heart rate of 115 beats per minute. Arterial pressure was 117/84 mmHg. Neurological status showed a marked neck rigidity with rigidly increased muscle tonus in all four limbs. The patient was somnolent with absent pathological reflexes, and grossly unperturbed sensation. The lung x-ray showed pneumonia on the left; a mildly marked cerebellar atrophy was detected by MRI.

The results of patient's serum laboratory tests (Table 1) showed: leukocytosis, elevated creatinine, C-reactive protein (CRP), creatine phosphokinase (CK), ferritin, lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and Myoglobin as well as low serum potassium. The presence of protein, ketone bodies, and bilirubin were found in urine. Uroculture and throat swab showed no bacterial growth. *Staphylococcus hominis*

Table 1. Patients' laboratory results

Parameter	Reference interval	Case 1	Case 2
Leukocytes, n*10^9/L	3.5-10.5	19.2	14.45
Creatinine, μmol/L	74-130	148	217
CRP, mg/L	0-5	102.12	45.54
Serum potassium, mmol/L	3.5-5.5	2.9	3.9
CK, U/L	15-180	8675.0	1931.0
Ferritin, ng/ml	30-400	1016.0	2016.0
LDH, U/L	135-225	665.0	1055.0
Myoglobin, ng/mL	25-70	>4102.0	>4102.0
AST, U/L	5-40	233.8	210.7
ALT, U/L	5-40	75.7	66.9
Serum WNV IgM, ELISA	<1.1	4.6	(+)
Serum WNV IgM, CLIA	<1.1	10.7	(+)
Serum WNV IgG, ELISA	<1.1	(–)	(–)
CSF WNV IgM, ELISA		(+)	
CSF WNV IgG, ELISA		Borderline	
CSF WNV PCR		(–)	
Urine WNV PCR		(–)	(+)
CSF leukocytes, n*10^6/L	<5	3	12
CSF protein, g/L	0.15-0.45	0.678	0.996

was isolated from haemoculture.

Serological tests revealed WNV specific IgM antibodies: 4.6 by ELISA and 10.7 by Chemiluminescence immunoassay (CLIA) (reference value < 1.1), and negative WNV IgG. The remaining specific serology tests were negative except for a positive Lyme disease IgG.

The Arbovirus Reference Laboratory at the National Center for Infectious and Parasitic Diseases (NCIPD) detected WNV specific IgM antibodies in serum and CSF and borderline IgG by ELISA; PCR in urine and CSF were negative. Cerebrospinal fluid analysis showed $3x10^6$/L leukocytes (reference values < $5x10^6$) and slightly elevated protein: 0.678g/L (reference interval 0.15-0.45).

Six days after the admission, the patient was intubated and placed on assisted pulmonary ventilation due to a marked respiratory failure and a comatose state. Despite the resuscitative measures, he ended lethally with the presentation of acute cardiovascular and respiratory failure.

Case 2

A 72-year-old man presented with a history of fever up to 38.5°C and lower dyspeptic syndrome lasting for one week. He was treated by his general practitioner with cefuroxime as an outpatient. After COVID-19 infection in 2021, the patient had a residual tremor of the right hand, mainly on purposive movements. Arterial hypertension was reported as a concomitant disease. Since two days the tremor had increased, and also appeared in the other arm, head and tongue, the patient developed severe adynamia to inability to walk, worsening of the general condition, and confusion, due to which the patient was admitted to the hospital. On the following day the patient became inadequate, disoriented, difficult to contact, with aphasia, low oxygen saturation and was transferred to the ICU. Initial assessment revealed a progressive general cerebral manifestation to coma, acute renal failure, and development of severe respiratory failure, necessitating intubation and placing on assisted pulmonary ventilation.

Lung X-ray showed stasis and small pleural effusions, with the subsequent development of bilateral pneumonia. Contrast computer tomography scan of the brain showed evidence of cerebral atrophy and long dated focus of lacunar ischemia in the right middle cerebral artery basin. ECG evidenced absolute arrhythmia and atrial fibrillation. Neurological status showed no meningoradicular irritation syndrome; craniocerebral nerves had no abnormalities; both pupils were isocoric, equally responsive to light. Static tremor of both hands was observed and tendon-anterior reflexes were generally attenuated for all four limbs.

Serum laboratory tests showed elevated C-reactive protein (CRP), LDH, myoglobin, ferritin; creatinine, AST and ALT (Table 1). Uroculture, hemoculture, and throat swab showed no bacterial growth. CSF showed elevated protein and mild pleocytosis.

Serological examination demonstrated specific IgM antibodies to West Nile virus (CLIA), and negative IgG. Specific WNV IgM antibodies in serum were confirmed at the Reference Arbovirus Laboratory of NCIPD. The PCR for West Nile virus in urine was positive. PCR test of CSF for Meningitis and Encephalitis Panel (S. pneumoniae, H. influenzae, Mycoplasma pneumoniae, Escherichia coli, Listeria monocytogenes, Neisseria meningitides, Streptococcus agalactiae, Enterovirus, Herpes simplex 1/2, Human herpes virus 6, Human parechovirus, Varicella zoster virus, Criptococcusneoformans, Streptococcus pyogenes) was negative.

Despite the treatment, the patient ended lethally on the 13th day after the admission with symptoms of cerebral edema, acute cardiovascular and respiratory failure.

DISCUSSION

In our article, both patients had encephalitis, which was a diagnostic challenge because all tests for the usual agents responsible for encephalitis were negative. Initial CSF examinations revealed mild proteinuria in both cases and mild pleocytosis in the second case. MRI showed no specific changes in the first case and was not feasible in the other case. In both cases, the diagnosis was made based on the risk factors, the season (August), symptoms, and laboratory findings. Confirmation of WNV aetiology was performed at NCIPD by ELISA and RT-PCR in serum, urine, and CSF. Both patients had not visited endemic areas and the WNV infection occurred in Sofia, making it a consideration in the differential

diagnosis of a neuroinfection.

While waiting for the results of the serological, CSF, and imaging tests empirical therapy for potential bacterial and viral meningoencephalitis was initiated with Ceftriaxone, Vancomycin and Acyclovir as well as symptomatic and causative anti-oedema therapy (dexamethasone, hepatoprotectors, diuretics, glucose-electrolyte solutions). After the positive result for WNV, the antiviral treatment was changed and Ribavirin was added to the therapy. Unstable hemodynamics was controlled with constant infusion of catecholamines.

These two reported clinical cases indicate that in advanced age and in the presence of comorbidities, WNND can have a severe clinical course and fatal ending. The mortality rate among patients can be as high as 17% [14, 15].

CONCLUSION

WNV infection is associated with considerable morbidity, particularly among the older individuals. Suspicion should be upheld in febrile patients with encephalitis or aseptic meningitis of unknown aetiology, admitted to the ICU. This is particularly important during the summer season when the disease can be spread by mosquitoes. More research is needed to identify the factors predicting a critical course requiring intensive care.

REFERENCES

1. Factsheet about West Nile virus infection. ECDC. Online: https://www.ecdc.europa.eu/en/west-nile-fever/facts. Last access: 01.11.2024
2. West Nile virus infection - Annual Epidemiological Report for 2019. Stokholm: ECDC; 2021. Online: https://www.ecdc.europa.eu/sites/default/files/documents/AER-WNV-infection-2019.pdf. Last access: 01.11.2024
3. Argirova P, Kalchev Y, Vasilev P, Christova I, Stoycheva M, Murdjeva M. West Nile virus meningoencephalitis and cytokines analysis: review and case report. Biotechnol Biotechnol Equip. 2024; 38(1):2358991. https://doi.org/10.1080/13102818.2024.2358991
4. Baymakova M, Trifonova I, Panayotova E, Dakova S, Pacenti M, Barzon L, Lavezzo E, Ramshev K, Plochev K, Christova I. West Nile virus neuroinvasive disease: The first confirmed case in Bulgaria. Int J Infect Dis. 2016; 53:150. https://doi.org/10.1016/j.ijid.2016.11.368
5. Kotsev S, Christova I, Pishmisheva-Peleva M. West Nile Fever - Clinical and Epidemiological Characteristics. Review of the Literature and Contribution with Three Clinical Cases. Fol Med (Plovdiv). 2020; 62(4):843-850. https://doi.org/10.3897/folmed.62.e51225
6. Rossi SL, Ross TM, Evans JD. West Nile virus. Clin Lab Med. 2010; 30(1):47-65. https://doi.org/10.1016/j.cll.2009.10.006
7. Gyure KA. West Nile virus infections. J Neuropathol Exp Neurol. 2009; 68(10):1053-60. https://doi.org/10.1097/NEN.0b013e3181b88114
8. Hayes EB, Komar N, Nasci RS, Montgomery SP, O'Leary DR, Campbell GL. Epidemiology and transmission dynamics of West Nile virus disease. Emerg Infect Dis. 2005; 11(8):1167-73. https://doi.org/10.3201/eid1108.050289a
9. Sambri V, Capobianchi M, Charrel R, Fyodorova M, Gaibani P, Gould E, Niedrig M, Papa A, Pierro A, Rossini G, Varani S, Vocale C, Landini MP. West Nile virus in Europe: Emergence, epidemiology, diagnosis, treatment, and prevention. Clin. Microbiol. Infect. 2013; 19:699–704. https://doi.org/10.1111/1469-0691.12211
10. Petersen LR, Brault AC, Nasci RS. West Nile virus: review of the literature. JAMA. 2013; 310(3):308-15. https://doi.org/10.1001/jama.2013.8042
11. Sejvar JJ, Curns AT, Welburg L, Jones JF, Lundgren LM, Capuron L, Pape J, Reeves WC, Campbell GL. Neurocognitive and functional outcomes in persons recovering from West Nile virus illness. Journal of neuropsychology. 2008; 2(2):477-99. https://doi.org/10.1348/174866407x218312
12. Sejvar JJ, Haddad MB, Tierney BC, Campbell GL, Marfin AA, Van Gerpen JA, Fleischauer A, Leis AA, Stokic DS, Petersen LR. Neurologic manifestations and outcome of West Nile virus infection. JAMA. 2003; 290(4):511-5. https://doi.org/10.1001/jama.290.4.511
13. Montgomery RR. Age-related alterations in immune responses to West Nile virus infection. Clin Exp Immunol. 2017; 187(1):26-34. https://doi.org/10.1111/cei.12863
14. Danis K, Papa A, Theocharopoulos G, Dougas G, Athanasiou M, Detsis M, Baka A, Lytras T, Mellou K, Bonovas S, Panagiotopoulos T. Outbreak of West Nile virus infection in Greece, 2010. Emerg Infect Dis. 2011; 17(10):1868-72. https://doi.org/10.3201/eid1710.110525
15. Pervanidou D, Detsis M, Danis K, Mellou K, Papanikolaou E, Terzaki I, Baka A, Veneti L, Vakali A, Dougas G, Politis C, Stamoulis K, Tsiodras S, Georgakopoulou T, Papa A, Tsakris A, Kremastinou J, Hadjichristodoulou C. West Nile virus outbreak in humans, Greece, 2012: third consecutive year of local transmission. Euro Surveill. 2014; 19(13):20758. https://doi.org/10.2807/1560-7917.es2014.19.13.20758
16. DeBiasi, R.L. West Nile Virus Neuroinvasive Disease. Curr Infect Dis Rep. 2011; 13, 350–359. https://doi.org/10.1007/s11908-011-0193-9
17. Gorchakov R, Gulas-Wroblewski BE, Ronca SE, Ruff JC, Nolan MS, Berry R, Alvarado RE, Gunter SM, Murray KO. Optimizing PCR Detection of West Nile Virus from Body Fluid Specimens to Delineate Natural History in an Infected Human Cohort. Int J Mol Sci. 2019; 20(8):1934. https://doi.org/10.3390/ijms20081934
18. Lanciotti RS, Kerst AJ. Nucleic acid sequence-based amplification assays for rapid detection of West Nile and St. Louis encephalitis viruses. J Clin Microbiol. 2001; 39(12):4506-13. https://doi.org/10.1128/JCM.39.12.4506-4513.2001
19. Tyler KL, Pape J, Goody RJ, Corkill M, Kleinschmidt-DeMasters BK. CSF findings in 250 patients with serologically confirmed West Nile virus meningitis and encephalitis. Neurology. 2006; 66(3):361-5. https://doi.org/10.1212/01.wnl.0000195890.70898.1f
20. Brilla R, Block M, Geremia G, Wichter M. Clinical and neuroradiologic features of 39 consecutive cases of West Nile Virus meningoencephalitis. J Neurol Sci. 2004; 220(1-2):37-40. https://doi.org/10.1016/j.jns.2004.01.013
21. Ali M, Safriel Y, Sohi J, Llave A, Weathers S. West Nile virus infection: MR imaging findings in the nervous system. AJNR Am J Neuroradiol. 2005; 26(2):289-297.
22. Petropoulou KA, Gordon SM, Prayson RA, Ruggierri PM. West

Nile virus meningoencephalitis: MR imaging findings. AJNR Am J Neuroradiol. 2005; 26(8):1986-95.
23. Loginova SIa, Borisevich SV, Pashchenko IuA, Bondarev VP. In Russian [Ribavirin prophylaxis and therapy of experimental West Nile fever]. Antibiot Khimioter. 2009; 54(11-12):17-20.
24. Odelola H. A. Antiviral activity of Virazole on replication of viruses isolated in Nigeria. Current Chemotherapy / Siegenthaler W., Lathy R. eds.Washington: American Society of Microbiology. 1978; 1: 3343—3345.
25. Jordan I, Briese T, Fischer N, Lau JY, Lipkin WI. Ribavirin inhibits West Nile virus replication and cytopathic effect in neural cells. J Infect Dis. 2000; 182(4):1214-7. https://doi.org/10.1086/315847
26. Anderson JF, Rahal JJ. Efficacy of interferon alpha-2b and ribavirin against West Nile virus in vitro. Emerg Infect Dis. 2002; 8(1):107-108. https://doi.org/10.3201/eid0801.010252
27. Ben-Nathan D, Lustig S, Tam G, Robinzon S, Segal S, Rager-Zisman B. Prophylactic and therapeutic efficacy of human intravenous immunoglobulin in treating West Nile virus infection in mice. J Infect Dis. 2003; 188(1):5-12. https://doi.org/10.1086/376870
28. Konkolova E, Dejmek M, Hřebabecký H, Šála M, Böserle J, Nencka R, Boura E. Remdesivir triphosphate can efficiently inhibit the RNA-dependent RNA polymerase from various flaviviruses. Antiviral Res. 2020; 182:104899. https://doi.org/10.1016/j.antiviral.2020.104899
29. Radoshitzky SR, Iversen P, Lu X, Zou J, Kaptein SJF, Stuthman KS, Van Tongeren SA, Steffens J, Gong R, Truong H, Sapre AA, Yang H, Xie X, Chia JJ, Song ZJ, Leventhal SM, Chan J, Shornikov A, Zhang X, Cowfer D, Yu H, Warren T, Cihlar T, Porter DP, Neyts J, Shi PY, Wells J, Bilello JP, Feng JY. Expanded profiling of Remdesivir as a broad-spectrum antiviral and low potential for interaction with other medications in vitro. Sci Rep. 2023; 13(1):3131. https://doi.org/10.1038/s41598-023-29517-9
30. Tunkel AR, Glaser CA, Bloch KC, Sejvar JJ, Marra CM, Roos KL, Hartman BJ, Kaplan SL, Scheld WM, Whitley RJ; Infectious Diseases Society of America. The management of encephalitis: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis. 2008; 47(3):303-27. https://doi.org/10.1086/589747