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Case Report

West Nile Virus Encephalitis in Haematological Setting: Report of Two Cases and a Brief Review of the Literature

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Abstract. West Nile virus is a zoonotic agent causing life-threatening encephalitis in a proportion of infected patients. Older age, immunosuppression, and mutations in specific host genes (e.g., CCR5 delta-32 mutation) predispose to neuroinvasive infection. We report on two cases of severe West Nile encephalitis in recently-treated, different-aged, chronic lymphocytic leukemia patients. Both patients developed high-grade fever associated with severe neurological impairment. The younger one harboured germ-line CCR5 delta-32 mutation, which might have played a role in the pathogenesis of its neuroinvasive manifestations.

Keywords: West Nile Virus, Encephalitis, Chronic Lymphocytic Leukemia, CCR5.

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Introduction. West Nile virus (WNV) infection is a zoonotic disease first recognized in Europe in the 1960s.[1] Although the virus can also be transmitted through blood transfusion or organ transplantation, humans are mostly infected by *Culex* mosquitos in the transmission season, typically lasting from July to October.[2] As compared to the previous 4 years, WNV infections have sharply increased in Europe in 2018,[3] probably due to circumstances favouring mosquito breeding and propagation. While 80% of infected patients are asymptomatic, some of them experience symptoms ranging from low-grade fever to severe neurological illness.[2] Several risk factors for central nervous system involvement have been identified, among which older age,[4] impaired adaptive immunity, and reduced chemokine receptor 5 (CCR5) expression appear pivotal.[5] At least the first two conditions are frequently shared among patients affected by chronic lymphocytic leukemia (CLL), an indolent B-cell lymphoproliferative disorder characterized by slowly progressive clonal expansion in primary and secondary lymphoid organs, and associated with an abnormal T helper cell profile.[6]

Here we describe two cases of severe WNV encephalitis occurring in recently-treated CLL patients in August 2018. Both cases have been recorded during the infection spike, taking place in Eastern Italy in the same period.

Case Report. (*a*) A 53-year-old man was diagnosed with Rai II/Binet B CLL in September 2014. Immunoglobulin variable region heavy chain genes (IGHV) were unmutated, fluorescent in situ hybridization (FISH) for common cytogenetic abnormalities was unremarkable and TP53 status was wild type. Because of massive symptomatic splenomegaly, he underwent Rituximab-Fludarabine-Cyclofosfamide treatment in November 2017 through April 2018, achieving partial remission. In August 2018, he was hospitalized with high-grade fever and

fatigue, without rash or meningismus. He denied any recent travel, sick contacts, or blood transfusion. Despite wide-spectrum antibiotics, his mental status and weakness rapidly worsened, also developing dizziness and ataxia. White blood cells count was 4500/μL, with moderate lymphopenia (600/μL). Analysis of lymphocyte subpopulation displayed an almost total absence of B cells and a reduced number of total T (560/μL) and NK cells (16/μL), with marked reduced T CD4/CD8 ratio (0.16, normal range 1.15-2.84). There was hypogammaglobulinemia (IgG 3.0 g/L) due to previous treatments. His cerebrospinal fluid (CSF) was clear with normal opening pressure, hyperprotidorrachia (65 mg/dL), average glucose, mild pleocytosis (10 leukocytes/μL) and negative bacterial and fungal cultures. Flow cytometric analysis did not show leukemic involvement. Magnetic resonance imaging (MRI) returned negative for both focal and diffuse signal alterations, whereas electroencephalography (EEG) showed diffuse slow wave activity without paroxysm. Within 3 days, he became poorly responsive to verbal stimuli and diffusely hyporeflexic. Because of the clinical suspect of viral encephalitis, Acyclovir treatment and immunoglobulins supplementation were promptly started without improvement in his neurological condition. Polymerase chain reaction (PCR) for WNV returned positive in both serum and urine, with no detectable specific serum antibodies likely due to hypogammaglobulinemia. All the other virological tests concerning neurotropic viruses, performed on CSF and serum, were negative. On hospital day 16, the patient's cognitive performance began to improve, and he gradually recovered his motor functions as well as osteo-tendon reflexes. Finally, he was discharged and transferred to a rehabilitation clinic for a full recovery. We searched for anomalies in CCR5. Sequencing of *Ccr5* gene led to identifying CCR5 delta-32 mutation in heterozygosis.

(*b*) An 85-year-old woman was diagnosed with Rai III/Binet C CLL in June 2014. For progressive and symptomatic anemia due to bone marrow failure, the patient underwent treatment with Chlorambucil and Rituximab in 2016, achieving a temporary improvement in her peripheral blood counts. In June 2018 she experienced a new worsening of anemia with high need of transfusion support. FISH and TP53 mutational status were negative, but cytogenetic analysis using B-cell mitogens showed del(3) (p13p21) and del(14) (q24) in 8 metaphases out of 20. Because of progressive disease, the patient started the second line Ibrutinib treatment. However, she autonomously discontinued drug assumption due to poor gastrointestinal tolerance as soon as thirty days after beginning. At the end of July 2018, she was admitted to the emergence department with fever, myalgias, anorexia and worsening fatigue. Her blood cell counts showed moderate lymphocytosis (24000/μL) with mild anemia and thrombocytopenia, whereas C-reactive protein and procalcitonin were in the normal range. Empirical antibiotic therapy was initially administered. However, her neurological conditions rapidly worsened over the following days, developing psychomotor agitation with stereotyped afinalistic movements of the lower limbs and altered mental status. Cranial computed tomography scan was unremarkable, and EEG showed diffuse slowing of background activity without focal spikes. Diagnostic lumbar puncture was performed, with normal opening pressure, clear CSF, pleocytosis of 44 leukocytes/μL (mononuclear prevalent), hyperglycorrhachia (66 mmol/L) and hyperprotidorrachia (67 mg/dL). As there was the clinical suspicion of viral encephalitis, intravenous Acyclovir treatment was initially administered. Serum and CSF WNV IgM returned positive, whereas all the other microbiological tests performed on serum and CSF did not. Also, serum WNV PCR was positive. Despite supportive measures, the patient's state of consciousness progressively declined, becoming unresponsive to any external stimuli. She finally died sixteen days after her hospital admission. Sequencing of *Ccr5* gene revealed no alterations.

Sequencing method. Genomic DNA was extracted from 200 μL of whole blood by use of QIAamp DNA mini kit (Hilden, DE), according to with manufacturer's instructions. DNA was amplified by Platinum Taq DNA polymerase High Fidelity (Invitrogen, Carlsbad, CA) using primers flanking the site of the 32 base pair deletion: 5'-CGCATCAAGTGTCAAGTCCAATC-3' and 5'- TGTAAACTGAGCTTGCTCGCT-3' (M-Medical, Cornaredo, IT). PCR products were purified with FastGene extraction kit (Nippon Genetics, Tokyo, JP). The reverse PCR primer was then used for Sanger sequencing with GenomeLab DTCS quick start kit (Sciex, Framingham, MA) and CEQ 8000 Genetic Analysis System (Sciex) following the manufacturer instructions.

Discussion. Since 2008, Italy contribution to European WNV infections increased substantially, with peaks of cases reported in 2013 and 2015.[7] WNV is maintained in a continuous mosquito-bird cycle, wherein mosquitos are the vectors, and the birds are the reservoir. Humans, just like horses and other mammals, represent dead-end hosts and do not contribute to further spread of the infection.[2] West Nile and other viral zoonotic infections have long been identified as a relevant cause of morbidity in people bearing genetic or acquired immune deficiencies. In recent years, the rapid spread of WNV infection have been overlapped with the rising number of immunocompromised hosts, due to increased median life expectancy, more powerful anticancer strategies and widespread usage of

Table 1. Reported cases of WNV encephalitis in haematological patients

Author	N° of cases	Age (years)	Diagnosis	Last previous treatment	MRI	WNV Serology	Outcome	Ref
Morjaria	1	37	DLBCL	R-CHOP	Thalamic involvement	Negative	Death	8
Huang C	1	70	Intermediateg rade B-NHL	R-CHOP	NP	Negative	Death	9
Hollander H	1	81	CLL	Fludarabine	Thalamic involvement	Negative	Death	10
Mawhorter SD	1	47	FL	R-Fludarabine	Left-mesial temporal lobe increased T2 and FLARE hyperintensity. Hippocampal and thalamic involvement	Negative	Death	11
Hindo H	1	14	ALL	Manteinance chemotherapy (VCR, 6MP, prednisone)	Thalamic, midbrain and brain stem involvement	Positive	Death	12
Farnaes L	3	5/6/9	ALL	MTX-VCR/ MTX-6MP/ MTX-VCR-DMS-NEL	Thalamic involvement in all cases	Positive in all cases	Death in 1 case. Recover with neurological sequelae in the others	13
Honig A	1	57	B-NHL	R-chemotherapy	Negative	Negative	Recover with residual cognitive impairment	14
Robertson KB	3	46/41/62	NHL/AML/AML	BMT	Thalamic and cerebral peduncles involvement in 1 out of 3 cases	Positive in all cases	Death in 1 out of 3 cases	15
Hiatt B	1	41	T-NHL	BMT	Pontomedullary junction involvement	Negative	Death	16
Penn RG	1	57	FL	CMVP	Negative	Negative	Death	17
Espinoza-Gutarra MR	1	64	CLL	Ibrutinib	Negative	Positive	Recover	18

DLBCL: diffuse large B cell lymphoma; R: Rituximab; CHOP: Cyclophosphamide, Doxorubicine, Vincristine, Prednisone; NHL: non-Hodgkin lymphoma; CLL: chronic lymphocytic leukemia; NP: not performed; FLAIR: Fluid attenuation inversion recovery; FL: follicular lymphoma; ALL: acute lymphoblastic leukemia; VCR: vincristine; 6MP: 6-mercaptopurine; MTX: methotrexate; DMS: dexamethasone; NEL: nelarabine; BMT: bone marrow transplantation; AML: acute myeloid leukemia; CMVP: Cyclophosphamide, Mitoxantrone, Vincristine, Prednisone.

immunosuppressive and immunomodulatory drugs, also for autoimmune and other non-malignant conditions. In haematological setting, several cases of neuroinvasive WNV disease have been reported (**Table 1**).[8-18] They mainly comprise patients diagnosed with WNV encephalitis while on treatment for lymphoid malignancies or recently undergone bone marrow transplantation, even in paediatric age. Most of them were hospitalized due to high-grade fever and neurological symptoms ranging from coarse tremor and cerebellar ataxia to seizures and confusional state. About a half of cases showed thalamic involvement at MRI, with hyperintensity on T2-weighted imaging and altered FLAIR sequences.[8,10-13,15] Diagnosis of neuroinvasive disease was confirmed either by positive WNV PCR (in CSF or plasma)[8,9,10-14,16] or by positive serological tests (IgM),[12,13,15,18] in the presence of suggestive clinical features. Notably, all Rituximab-treated patients were diagnosed by PCR,[8,9,11,14] as serological tests always returned negative due to treatment-related B-cell impairment. However, clinicians may also be aware that viremic phase is short in humans,[16] thus negative molecular tests do not exclude the diagnosis. This makes even more challenging the diagnostic process in immunocompromised subjects. In them, the outcome was fatal for 8 out of 15 patients, and some survivors experienced severe neurological sequelae,[13,14] which often parallel cortical thinning and regional atrophy detected by neuroimaging studies.[19] None of them was evaluated for *Ccr5* gene status.

The two cases we described well recapitulate the main clinical and laboratoristic features of severe WNV infection. Both of them were affected by CLL and were recently treated in an outpatient setting with chemoimmunotherapy and Ibrutinib, respectively. To our knowledge, only two patients affected by WNV encephalitis previously diagnosed with CLL have been described in the literature.[10,18] Our patients presented with fever and went through a rapid deterioration of neurological conditions. C-reactive protein was not elevated, CSF analysis showed alterations consistent with blood-brain barrier (BBB) damage and neuroimaging was insignificant. This latter occurs in about 50% of patients affected by WNV encephalitis, the other half developing abnormal MRI findings, which commonly involve thalami, basal ganglia, mesial temporal structures, brain stem, and cerebellum.[20] In the first, Rituximab-treated, patient, the diagnosis was based on positive WNV PCR, as serology returned negative. The fatal outcome of the second patient was likely favoured by older age and female gender, usually associated with worse recovery from coma.[21]

Since most neuroinvasive WNV disease in non-haematological setting occur in the elderly,[2] the

development of such a severe clinical picture in the first, young patient prompted us to investigate the presence of *Ccr5* delta-32 mutation as an additional risk factor for neuroinvasive infection.[22] CCR5 is the G protein-coupled receptor binding to three CC chemokines, namely CCL3, CCL4 and CCL5, involved in T lymphocyte trafficking through the BBB. It is mainly expressed by T helper 1 subset upon antigen recognition, whereby coupling the amplification of the inflammatory response with the appropriate environmental context.[23,24] By sequencing the *Ccr5* gene, we found the patient was heterozygous for the delta-32 mutation, which generates a truncated form of CCR5 persistently retained within the endoplasmic reticulum, thus lowering its surface expression. Of note, delta-32 CCR5 has been reported to act as a dominant negative mutant, further reducing the cell sensing for CCR5 cognate ligands.[25] Therefore, impairment of CCR5-based chemotactic system, along with low T CD4 cell count due to previous chemotherapy, may have played a non-negligible role in the pathogenesis of his neuroinvasive WNV infection. We speculate that *Ccr5* genotyping might be of some importance to identify immunocompromised patients particularly at risk for life-threatening neurological complications. Effective preventive strategies, mostly still to be uncovered, might be primarily directed to them.

Conclusions. The two cases we reported highlight some general considerations. First, the rapid spread of WNV infection should induce clinicians to take it into account within the diagnostic workup of immunocompromised patients with fever and neurological symptoms, especially in summer months. In haematological setting, outpatient regimens are even likely to increase the risk of neuroinvasive disease, and diagnosis may be difficult in those patients treated with monoclonal antibodies blunting humoral response. Then, assessment of genetic risk factors for severe WNV infection, such as *Ccr5* delta-32 mutation, may be useful to direct future preventive or pre-emptive strategies. However, supportive measures remain the only way to face the disease so far, and fatal outcome is still frequent.

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