Molecular detection and isolation of West Nile virus from a human case in northern Greece, 2013

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Abstract

In order to laboratory confirm the first suspected West Nile fever case in 2013 in northern Greece, a combination of serological molecular and culture methods were applied. It was shown that the causative West Nile virus strain belonged to lineage 2, and possessed the amino acid substitution H249P in the NS3 protein, as in previous years. The significance of this specific strain in Europe remains to be elucidated.

Keywords: 2013, Greece, lineage 2, West Nile fever, West Nile virus

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Introduction

West Nile virus (WNV) is a mosquito-borne flavivirus (genus Flavivirus, family Bunyaviridae) causing an asymptomatic or mild infection (West Nile fever) in humans, while in less than 1% of infections there are symptoms from the nervous system (West Nile neuroinvasive disease). Most WNV strains cluster into two major genetic lineages 1 and 2. Lineage 1, is responsible for sporadic cases and outbreaks in Africa, Asia, Australia and the Americas. Lineage 2, initially restricted to sub-Saharan Africa and Madagascar, emerged in 2004 in Europe, and caused a large outbreak of WNV infections in 2010 in Greece (WNV strain Nea Santa-Greece-2010) [1,2]. Genetic characterization of the strain showed that its closest genetic relationship was to the lineage 2 strain that had emerged in Hungary in 2004, and it was suggested that the increased virulence might be associated with the amino acid substitution H249P in the NS3 protein [3]. Since then, Greece has experienced outbreaks for three consecutive years (2010–2012) with 381 cases of West Nile neuroinvasive disease [4]. The geographical range of WNV in Europe is expanding and the virus is causing increasing numbers of epidemics/outbreaks associated with human morbidity and mortality [5]. Here, we report the laboratory-confirmed diagnosis of the first West Nile fever case observed in 2013 in northern Greece.

In mid-July 2013 a 73-year-old male resident of Thessaloniki, northern Greece, with a history of diabetes mellitus, coronary disease and dementia, was admitted to Agios Demetrios General Hospital in Thessaloniki, because of 4-day high fever (39.7°C), chills, severe headache, cough, nausea and vomiting. Upon admission the patient was disorientated. His blood pressure was 140/80 mmHg with a heart rate of 70 beats per minute. Main laboratory findings were: white blood cell count 14 000 cells/mL (neutrophils 72.6%, lymphocytes 17%, monocytes 9.9%); haematocrit 38.7%; platelets 108 000/mL; erythrocyte sedimentation rate 66 mm/h; C-reactive protein 3.2 mg/L (normal <3 mg/L); hyponatraemia 126 mmol/L (normal range 135–145 mmol/L); alanine transaminase 54 U/L; aspartate transaminase 65 U/L (normal range for both transaminases up to 35 U/L); and lactate dehydrogenase 409 U/L (normal range 140–280 U/L). No lumbar puncture was performed. The physical and neurological examination did not show any abnormalities, apart from reduced visual acuity due to cataract. Results of the chest radiography and electrocardiogram were normal, while a computed tomography scan of the brain showed generalized atrophy. The patient was started on antimicrobial drug therapy (intravenous ceftriaxone and azithromycin) for potential bacterial infection. Blood and urine cultures were negative. He continued to be febrile up to day 5 of hospitalization.

On the basis of the patient's clinical symptoms, and the several cases of WNV infection in northern Greece during the last 3 years, WNV infection was suspected. A serum sample from the patient was sent to the Hellenic Reference Centre for Arboviruses in Aristotle University of Thessaloniki for the detection of WNV IgM and IgG antibodies. Using commercial ELISA kits (WNV IgM capture DxSelect™ and WNV IgG DxSelect; Focus Diagnostics Inc., Cypress, CA, USA), WNV IgM antibodies were detected, but IgG antibodies were not detectable. A second serum sample, together with blood and urine samples, was taken on day 7 after admission (11th day of illness). High levels of both IgM and IgG antibodies were detected in the second sample, which was also tested by

plaque reduction neutralization test and >90% reduction was observed ($PRNT_{90}$).

A real-time RT-PCR [6] was applied on genetic material extracted from the plasma and urine samples of the patient (taken on the 11th day of illness), which gave negative results for the plasma sample but positive for the urine sample, with Ct value of 26.95. WNV RNA detection in urine has been proposed as a diagnostic method for WNV infection, because it is detectable at a higher viral load and for longer time in urine than in plasma and it has been successfully followed as routine testing for the diagnosis and follow-up of patients with WNV infection [7,8].

Upon receipt of the urine sample, 1 mL of 1 : 10 urine dilution was inoculated in Vero E6 cells, and flasks were observed daily for the presence of cytopathic effects, which were seen on day 3 after inoculation. Real-time PCR on the genetic material extracted from the cell supernatant gave positive results with Ct 19.60. We continued the propagation of the virus up to the sixth passage. Spot-slides prepared with the infected cells gave strong immunofluorescence with a known WNV IgG-positive serum. Hence, although the patient had already high titres of IgG and IgM antibodies, he continued to have viruria. To check whether the WNV strain of 2013 (North Greece-2013) possesses the H249P substitution in the NS3 protein, we applied an RT-nested PCR, with previously designed primers specific for this genome region [9]. Nucleotide sequencing analysis showed 100% identity with the Nea Santa-Greece-2010 strain, suggesting that the WNV strain of 2013 possess the H249P substitution (GenBank accession number KF537659).

The condition of the patient improved on day 7 of hospitalization, however, he was still complaining of headache. The haematological and biochemical parameters returned to normal and the patient was discharged on day 11 of hospitalization without any sequelae.

In the present study we report all the laboratory methods applied at a reference centre to three types of samples taken from the first suspected WNV case in northern Greece in 2013 in order first to confirm the diagnosis and then to gain an insight into the WNV strain that is causing clinical cases at the start of the WNV season. Although a lot of laboratory effort is needed to complete the procedure in the required time, it provides useful data for the WNV epidemiology in the country. For cases that might follow, serology and real-time RT-PCR are sufficient for WNV diagnostics, while $PRNT_{90}$ could be applied on batches of samples once or twice per month as a confirmatory test of the probable cases.

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