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Case Report

Diagnostic challenges in *Flaviviridae* infections: first human case of Usutu virus aseptic meningitis in an immunocompetent subject in Italy

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ABSTRACT

Objectives: Usutu virus (USUV) is a mosquito-borne Orthoflavivirus. Human USUV infections are rare and are often asymptomatic or mild; however, neuroinvasive manifestations such as meningoencephalitis have occasionally been reported.
Design or Methods: Diagnosis of USUV infection is challenging due to serological cross-reactivity with other flaviviruses, particularly West Nile virus, which often shares the same geographical distribution, as well as the lack of rapid and accurate serological and molecular diagnostic tools.
Results: Here, we report the first case of aseptic meningitis caused by USUV in an immunocompetent subject in Italy.
Conclusion: Highlighting the complex microbiological diagnostic pathway required to achieve an etiological diagnosis.

Background

Usutu virus (USUV) is an Orthoflavivirus belonging to the *Flaviviridae* family, transmitted mainly by mosquitoes of the *Culex* genus, with wild birds representing the reservoir of infection [1]. In humans, who are accidental hosts, the infection is mostly asymptomatic but can manifest with fever, rash, and in more severe cases, neurological involvement [2]. The first evidence of USUV circulation in Italy dates to 1996, following investigations into wild birds found dead in the provinces of Florence and Pistoia (Tuscany, central Italy) [3]. To date, eight different lineages have been identified, among which the Europa-2 lineage is the most widespread on the European continent [4]. The first documented human case of USUV meningoencephalitis was described in 2009 in a patient with diffuse lymphoma [5]. In subsequent years, further clinical cases have been reported in various regions of central and northern Italy [6–8]. At the same time, entomological and ornithological surveillance carried out in recent years has confirmed the active and persistent circulation of the virus in various areas of Tuscany [9]. In this context, we present the first documented case of symptomatic USUV infection in Tuscany, Italy.

Case presentation

A 71 year old immunocompetent man with a history of paroxysmal atrial fibrillation, arterial hypertension, and asymptomatic hyperuricemia, receiving flecainide, perindopril, and allopurinol for these conditions, was admitted to the Emergency Department of the University Hospital of Siena (Tuscany, Italy) in mid-September because of a 10 day history of fever associated with worsening left temporoparietal headache and severe myalgia of the lower limbs.

During the medical interview, the patient denied recent travel abroad and tick bites but reported multiple mosquito bites, consistent with the late summer season. Blood tests revealed normal white and red blood cell counts, platelet levels, and liver and renal function tests, with a mild increase in inflammatory markers (C-reactive protein 0.65 mg/dl; normal values <0.50 mg/dl).

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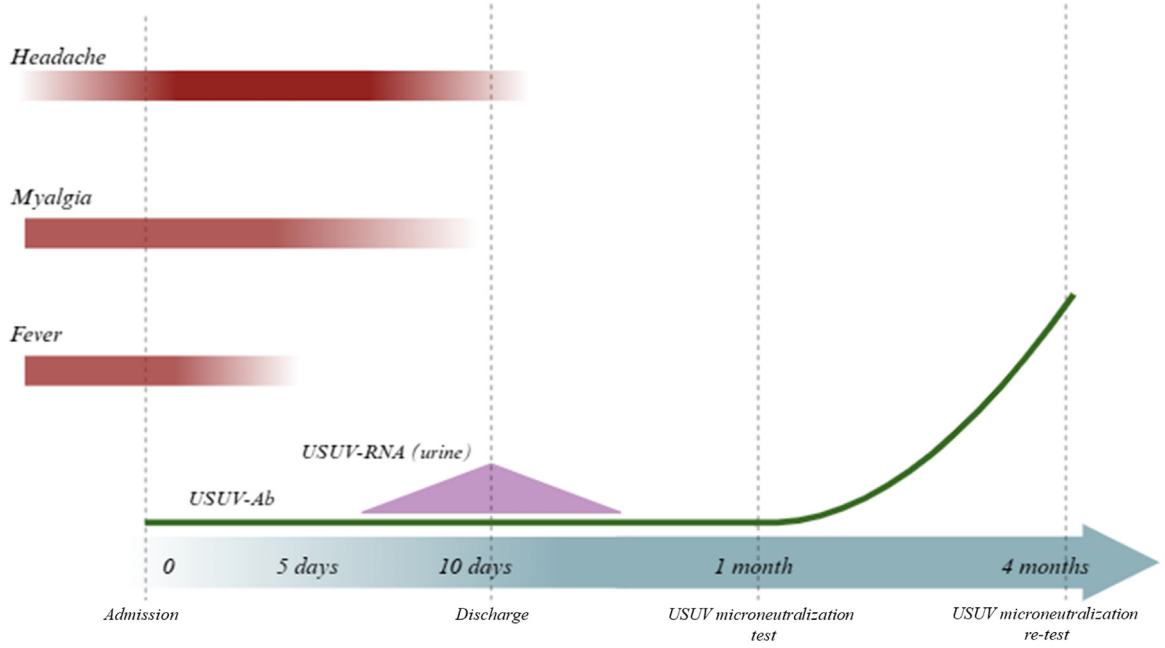

Figure 1. Timeline of the patient's clinical course and qualitative laboratory findings. Red bars indicate the onset and duration of clinical symptoms. The triangle marks the period of viral RNA detection by RT-PCR, while the green line represents the kinetics of serum antibodies.

Blood and urine cultures were negative. No relevant findings were detected on abdominal ultrasound, chest X-ray, or brain CT scan.

The patient was admitted to the Infectious and Tropical Diseases Department for further investigation, and anti-inflammatory therapy with ibuprofen was initiated, resulting in resolution of myalgia. Due to persistence of headache, a lumbar puncture was performed on day +5. Cerebrospinal fluid (CSF) analysis showed glucose 55.0 mg/dl (with concomitant blood glucose of 90.0 mg/dl), mild hyperproteinorrachia (protein 89.0 mg/dl; normal values: 20-40 mg/dl), and 11 leukocytes/mm^3 (normal values: 0-5 cells/mm^3), predominantly mononuclear cells (81.8%). CSF cultures were negative, as was molecular testing for neurotropic pathogens, including Adenovirus, CHIKV, CMV, EBV, HSV-1, HSV-2, VZV, Enterovirus, USUV, West Nile virus (WNV), TBE virus, TOSV, and *Borrelia spp*.

Molecular investigations using reverse transcription polymerase chain reaction (RT-PCR) to detect WNV in plasma and urine were negative.

Serological testing performed using VirClia Lotus Monotests (Vircell S.L., Granada, Spain) revealed high titers of anti–WNV IgM in the absence of IgG, suggesting a recent arboviral infection. Upon repeat testing at day +20, anti-WNV IgM titers had decreased. Notably, anti–USUV IgM and IgG, assessed using an envelope-based ELISA (Euroimmun, Lübeck, Germany), were negative at both time points.

As the patient's clinical condition progressively improved, he was discharged on day +10 with a diagnosis of arboviral meningitis. Following the patient's discharge, further investigations were performed to establish a definitive etiological diagnosis. In this context, USUV infection was diagnosed by RT-PCR (cycle threshold value of 28) performed on a urine sample, using a protocol previously described [10]. Thereafter, nucleic acids were extracted from urine and subjected to sequencing with the Illumina Viral Sequencing Panel (Illumina S.r.l., Milan, Italy). The sequence exhibited high nucleotide identity (99.45%) to the reference USUV isolate ITA-055 (Acc. Number KX268471.1), classified as Europe 2 lineage. The partial sequence was deposited in GenBank under accession number PZ182085.

Given the persistence of anti-WNV IgM antibodies, a serum microneutralization test was performed, which showed a neutralizing titer <1:4. This result ruled out WNV infection and supported the presence of antibody cross-reactivity among flaviviruses. The envelope-based ELISA was repeated, yielding equivocal results. One month after admission, molecular testing for both WNV and USUV in plasma and urine samples was negative.

Four months later, a new serum sample was obtained from the patient and retested using the same commercial ELISA kit, revealing a high titer of anti-USUV IgG (ratio calibrator/sample 1.6; cut-off 1.1). Additionally, the serum microneutralization test showed a neutralizing titer of 1:180, thus definitively confirming the diagnosis of USUV infection. A summary of the clinical course is shown in Figure 1.

Discussion

This case describes an episode of acute arboviral infection with neurological involvement, characterized by a challenging diagnostic workup, particularly in distinguishing the etiological role of WNV and USUV, both members of the *Flaviviridae* family and known to exhibit frequent serological cross-reactivity.

USUV infection in humans can manifest with a wide spectrum of clinical presentations, ranging from asymptomatic infection to a mild flu-like illness characterized by fever, asthenia, and maculopapular rash, up to neuroinvasive disease, including meningoencephalitis, radiculoneuritis, or cranial nerve palsy [11]. The mortality rate has not been well established due to limited available data; however, it appears to be higher in elderly and immunocompromised individuals [8,12].

A diagnostic gap still exists in the rapid identification of USUV as the causative agent of acute illness. In severe clinical conditions, such as central nervous system infections, timely identification or exclusion of the etiological agent is essential to ensure appropriate clinical management. Several studies have reported the

absence of detectable anti-USUV antibodies during the early phase of infection, suggesting delayed kinetics of the immune response [13].

Consistently, in this case, no serological response was detectable during the first 21 days after symptom onset, whereas viral RNA was identified in urine within 2 weeks. The absence of detectable viral RNA in CSF and blood may reflect the short duration of viremia and the limited sensitivity of CSF PCR, depending on the timing of lumbar puncture. In contrast, urine may allow longer persistence of viral RNA and thus represent a useful specimen for molecular detection [14]. Although intrathecal IgM against USUV was not assessed, the combination of urine RT-PCR positivity, viral sequencing, exclusion of WNV by microneutralization, and subsequent seroconversion with neutralizing anti-USUV antibodies strongly supports the etiological diagnosis.

Beyond the individual case, this diagnostic complexity reflects a broader epidemiological scenario in Italy, where widespread environmental circulation of USUV among birds and mosquito vectors contrasts with the relatively limited number of reported human cases. This discrepancy suggests that human infection is frequently asymptomatic or misclassified as WNV infection. The two viruses share overlapping ecological niches, circulate in the same geographic areas, and rely on common *Culex* vectors, with documented stable co-circulation in central and northern Italy [15]. Recent increases in WNV activity and climate-driven changes favoring vector proliferation further amplify the likelihood of simultaneous exposure and contribute to diagnostic bias [16].

These findings highlight the need to establish a rigorous diagnostic workup that includes accurate, easy-to-perform, and rapid molecular tools for the identification of USUV in patients considered at high risk, such as those with suspected central nervous system infection, those living in or travelling from endemic areas, and during periods of vector activity, from spring to late autumn. In conclusion, considering that USUV is still circulating in certain European regions and that ongoing environmental changes may affect arthropod and reservoir dynamics, potentially leading to a sustained increase in arboviral infections in humans [17], a high level of clinical awareness is required. Early and accurate molecular diagnosis in clinical practice remains essential to better characterize the impact of this emerging virus.

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Ethical approval and informed consent

Ethical review and approval were not required for this manuscript. Written informed consent for the publication of this case was obtained from the patient.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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