Zika virus infections imported to Italy: Clinical, immunological and virological findings, and public health implications

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1. Why this case is important?

Zika virus (ZIKV) is an arbovirus belonging to the Flavivirus genus that was first isolated from a rhesus monkey in 1947 in Uganda [1]. Since its discovery, evidence of human infections has been reported from Egypt, several sub-Saharan African countries, as well as South-Eastern Asian countries, and Oceania (Yap island in the Federated States of Micronesia) [2]. Beginning at the end of 2013, a large outbreak affected several islands of the West Pacific [3,4]. Imported cases have been described in several non-endemic countries including Japan, Germany, Canada, Australia and United States [5–9].

ZIKV is transmitted by different species of Aedes mosquitoes. Non-human primates and possibly rodents play a role as reservoir [10,11]. Direct interhuman transmission has been reported to occur perinatal [12] through blood transfusion [13], and sexually [9]. Occupational transmission in the laboratory setting has also been reported [14]. Returning travelers with ZIKV infection developed symptoms up to 6 days after leaving endemic areas [5–9], while symptoms in the patient with the presumed sexually transmitted infection began 10 days after the sexual intercourse with the index case [9]. Only about 18% of human infections result in clinical manifestations [15]. Most common clinical signs and symptoms are maculopapular rash, low grade fever, arthralgia, myalgia, headache and conjunctivitis, while oedema, sore throat, cough, vomiting, and
haematospermia have been reported less frequently [8,9,15,16]. The symptoms usually resolve spontaneously after 3–7 days [15], but arthralgia may persist for up to 1 month [9]. Recently, a possible correlation between ZIKV infection and Guillain–Barré syndrome (GBS) has been proposed [17].

Information on typical laboratory alterations associated with ZIKV infection are scarce, but include leukopenia, thrombocytopenia [5], and slight elevation of serum lactate dehydrogenase, gamma-glutamyl transferase, and of inflammatory parameters (C-reactive protein, fibrinogen and ferritin) [7]. Despite clinical manifestations of ZIKV being usually mild and self-limiting, the infection should be regarded as a potential threat for temperate countries, where competent mosquito vectors [18] are widespread [19]. The predisposition of Mediterranean countries for autochthonous transmission of Aedes-borne arboviruses such as, chikungunya virus (CHIKV) and DENV has already been, clearly, demonstrated [20-24].

We here report two cases of laboratory confirmed ZIKV infection in Italian travelers returning from short-term holiday trips in French Polynesia.

2. Case reports

A married couple in their early 30s presented to the Infectious and Tropical Diseases Unit, Azienda Ospedaliero Universitaria Careggi, Florence (Italy) one day after their return from a holiday trip to French Polynesia (December 17, 2013 to January 4, 2014). Both patients had a slightly itchy maculopapular rash diffused on the face, trunk, arms and legs, accompanied by low grade fever (maximum temperature 37.8 °C), conjunctivitis, malaise, weakness, myalgia, arthralgia, ankle oedema, and axillary and inguinal lymphadenopathy. The woman had taken three tablets of acetylsalicylic acid before presentation, because of intense muscular pain.

Fever and the cutaneous rash resolved 2 days after presentation, while malaise, weakness, and myalgia lasted 1 week in both cases. Lymphadenopathy was present for 2 weeks. The woman had a slight gingival bleeding for a few days after initial presentation.

Because of the symptoms and the travel history of the patients to French Polynesia during the ongoing ZIKV outbreak, an acute ZIKV infection was suspected. Serum samples of the acute and convalescent phase and heparinized, whole blood samples were sent for further analyses to the Bernhard Nocht Institute for Tropical Medicine, WHO Collaborating Centre for Arbovirus and Haemorhagic Fever Reference and Research, Hamburg, Germany.

The results, reported in Table 1, confirmed acute ZIKV infection in both travelers. IgG and IgM seroconversion against ZIKV, by using an indirect immunofluorescence assay, was demonstrated in the two patients (Table 1).

ZIKV-specific real-time RT-PCR [7] with the AgPath-ID One-Step RT-PCR Kit (Life Technologies, Carlsbad, CA, USA) was performed according to the manufacturer’s instructions, and results were only positive for the female patient with ZIKV RNA load below 10^3 copies/mL (Table 1).

To determine the immunological status of ZIKV patients during the convalescent phase of the disease, we designed a multiparameter flow cytometry panel to evaluate cellularity of immune cells in the patients peripheral blood samples (Fig. 1A). Our data did not reveal any abnormality in ZIKV patients compared to healthy controls (Fig. 1A and B). Since dendritic cells (DCs) are primary infection targets for most mosquito-borne flaviviruses, we next sought to determine the functionality of blood DCs in convalescent ZIKV patients compared to healthy controls (Fig. 1C and D). To do so, we assessed the antigen uptake capacity of DCs, using fluorescent FITC-dextran beads, as surrogate antigens. Our data indicates that blood DCs from our ZIKV patients are equally effective for antigen capturing than DCs from healthy donors (Fig. 1C and D). Taken together, our data strongly suggests that recovery from ZIKV infection is associated with restoration of normal numbers of immune cells in the periphery as well as with normal function of antigen-presenting cells, presumably the primary targets of ZIKV infection.

3. Other similar and contrasting cases in the literature and discussion

We reported the first two cases of laboratory confirmed ZIKV infections imported to Italy, with direct detection of the viral RNA in the serum of one case. Italy is the European country most heavily infested with Aedes albopictus [19], a competent vector for DENV
Fig. 1. A: Gating strategy of peripheral blood leukocyte (PBL) subsets in Zika patient samples. A large gate was drawn in the light scatter plot to include all relevant leukocyte subsets. Viable single cells were further selected via singlets live/dead cell discrimination. B: Quantification of leukocyte populations in healthy control samples and Zika patients. C and D: PBLs from Zika-infected patients as well as control individuals were incubated with 20 μg/mL of fluorescent FITC-dextran beads for 60 min at either 37 or 4 °C. Analysis of bead uptake by dendritic cells was done by evaluation of the FITC median fluorescence intensity (MFI) of CD3−CD19−CD11c+HLA−DR+ cells. Upper panel (C) shows representative histogram plots, and lower panel (D) shows MFI quantification. Red blood cells were depleted, using BD Pharm Lysing Buffer (BD Biosciences), according to the manufacturer’s instructions. Single cell suspensions were obtained by using a 70 μm cell strainer. Fc receptors were blocked with CD16/CD32 Fc Block antibody (BD Biosciences) followed by staining with fluorochrome-conjugated antibodies. Cells were fixed and permeabilized, using Cytofix/Cytoperm buffer, from BD Biosciences in the presence of 4% formaldehyde. An LSR Fortessa instrument (BD Biosciences) was used for flow cytometry. Analysis of flow cytometry data was performed using FlowJo software (Treestar). Graphs were plotted with Graph Pad Prism software.

and CHIKV. A. albopictus has also been demonstrated to be a potentially suitable vector for ZIKV [18]. The arrival of viremic travelers with acute ZIKV infections in Mediterranean countries, where competent mosquito vectors are already present, pose a risk for the local immunologically naive and thus, vulnerable human population.

As the biting activity of A. albopictus in Italy is seasonal and estimated to range from June 15 to November 30 [25], the risk of introduction and local spread of ZIKV by mosquitoes was practically nil in our cases. Taking into account the seasonal synchronicity, the introduction and establishment of ZIKV and other A. albopictus transmitted arboviruses into Italy is more likely to be imported from a Northern Hemisphere country than from a region in the Southern Hemisphere (such as, Polynesia) [26,27].

Nevertheless, in the light of autochthonous DENV and CHIKV infections in France [22,28], Croatia [20], and Italy [23], attention should be paid to the possibility of the ignition of local ZIKV outbreaks.

Adequate public health preventive measures, such as, public education and mosquito bite prevention, should be implemented quickly after the diagnosis of an imported case.

Possible further control measures include, the “fiduciary isolation” of the patient during the viremic phase and perifocal vector control activities centered on the case’s residence including spraying adult mosquitoes and destruction of larval breeding sites [29].

In this perspective, the role of clinicians is crucial and includes the early diagnosis of imported arboviruses such as, ZIKV infection and the timely notification of public health authorities. Clinicians should be aware of current outbreaks in parts of the world that are popular tourist destinations. This is especially important for newly emerging and possibly devastating diseases with specific public health implications [30].

The proposed case definition for suspected ZIKV infection, currently used in French Polynesia, [31] can be used to identify suspected imported cases in travelers who develop compatible symptoms within 1–2 weeks after returning from endemic areas. Cross reactive DENV serology (IgG or IgM) during ZIKV infection, which has been described in several previously reported cases [7], may be carefully used as marker to identify subjects with ZIKV, since commercial serological tests for DENV are widely available. Taking into account possible cross-reactions among different virus belonging to the Flavivirus family when using current serological tests, an approach combining direct and indirect detection techniques, as well as neutralization assay for confirmation, should be utilized [7,9].
Finally, the reported cases highlight the need of improving pre-travel advice and consultation for travelers planning to visit countries, where various arboviruses are endemic. Such advice should include effective preventive measures of mosquito bites, and avoidance of the use of acetylsalicylic acid, which is contraindicated in suspected or confirmed dengue fever due to the increase risk of bleeding [32].

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Competing interests

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Ethical approval

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References