Lack of KIs virus DNA in plasma and cerebrospinal fluid in Italy

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Dear Sirs,

Satoh et al. recently screened 516 Japanese blood donors with PCR using primers constructed from the consensus domain of the helicase of positive-stranded RNA viruses. They reported a novel enveloped virus with a circular double-stranded DNA genome (tentatively named KIs virus, KIs-V) (Satoh et al., 2011) occurring in 36 out of the 100 hepatitis E (HEV) antibody–positive donors with elevated alanine aminotransferase (ALT) levels (>60 IU/L). More recently, Biagini et al. failed to find KIs-V in plasma from 576 French blood donors with unknown HEV serostatus and unknown ALT values (Biagini et al., 2012). Based on an HEV seroprevalence of 3-52% in France, the authors suggested an uncommon frequency of KIs-V infection in healthy persons in France. To date, no information has been available on the prevalence of KIs-V DNA in Italy. In the present paper, we analyzed KIs-V in 242 plasma samples of blood donors, transplant recipients, and patients with chronic viral infections, and in 52 cerebrospinal fluid (CSF) samples of patients with different neurological disorders. Informed consent was obtained from all patients and the study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its amendments.

Viral DNA extraction was carried out on 200 µl of plasma or 200 µl of CSF by using QIAamp DNA blood kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Extracted nucleic acids were amplified for KIs-V DNA with the nested PCR protocol developed by Satoh et al. (2011) and used for screening Japanese blood donors. The first and second PCR rounds were designed on 458 and 304 nt-length fragments, respectively. To validate the amplification process, positive controls obtained from plasma dilutions of a synthetic template corresponding to the target sequence were run in each PCR. PCR sensitivity was less than 5 copies of target sequence.

Fourteen liver and 16 kidney and/or pancreas transplant recipients were tested before transplantation and at the time after transplantation when viremia levels of TTV were highest, TTV having been validated by our group and others as a marker of functional immune deficiency (Focosi et al., 2014). None of the samples tested positive for KIs-V. At the same time, we also tested 79 healthy blood donors. Since determination of ALT is a mandatory part of blood donation according to Italian law we could establish that only 2 donors had ALT values >60 IU/L but in any case <80 IU/L: all of them tested negative for KIs-V. No information on HEV status was available and HEV seroprevalence studies are limited in Italy (Arends et al., 2014). However regional studies show prevalences ranging from 2.9% to 8.8% (Masia et al., 2009). We also tested 50 HIV-positive patients, 41 HCV-positive patients, and 42 HBV-positive patients. None of the samples tested positive for KIs-V. Finally, cerebrospinal fluid from 52 patients with different neurological disorders was...
also tested. All these samples were negative for KIs-V DNA. Thus, although we cannot rule out the possibility that KIs-V circulates in Italy at a very low level and genetically different from the virus found in Japanese population, the results seem to demonstrate a very low prevalence of this novel virus in the Italian population. While the implication of KIs-V in human health remains under debate, extensive regional surveys will help to elucidate the geographical spread of KIs-V and to understand the natural history of the infection in human beings.

REFERENCES


