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Enterovirus D68 diagnosed in severe respiratory and neurological illness in children during 2015–2016 season in Portugal

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Background: Enterovirus D68 (EV-D68) was first isolated in 1962, and since then associated with respiratory illness. The report of severe respiratory and neurological disease including deaths associated to EV-D68 in United States and Canada during August 2014 highlighted the need of epidemiological information regarding EV-D68 circulation. In Europe information was scarce, available only for few countries. In Portugal there was no data available and was critical to know the epidemiology of EV-D68, especially in children hospitalized with severe respiratory or neurological disease. This study aims to identify EV-D68 in Enterovirus positive respiratory samples in children under 18 with clinical diagnosis of severe respiratory infection or neurological illness.

Methods: During 2015/16 winter season, between November/2015 and March/2016, 29 EV positive cases were reported to the National Influenza and Other Respiratory Virus Reference Laboratory (NIC) by two hospitals located in Lisbon and Setubal districts. EV diagnosis was performed in hospitals by biomolecular methods using commercial kits (real time multiplex-PCR, FTD Respiratory pathogens 21 and CLART Pneumovir, Genomica, respectively). EV-D68 was diagnosed by an in house real-time PCR [1]. Virus isolation in RD cell line and phylogenetic analysis of the VP1/VP3 genomic regions will enable the identification of genetic groups in circulation. All samples were irreversibly anonymized. Demographic and clinical data were collected.

Results: EV-D68 was confirmed in 20 respiratory samples previously positive for EV (69%; 20/29). Samples were collected from children with age ranging from 2 months to 6 years old, both genders (9 female; 11 male) with diagnosis of severe respiratory or neurological illness. Eighteen cases were hospitalized (90%; 18/20). Bronchiolitis and pneumonia were the most frequently reported diagnosis, corresponding to 70% (14/20). Two cases have neurologic diagnosis. EV-D68 was identified throughout all study period with the higher number of positive cases detected during January 2016, in week 3. Virus isolation and genetic characterization are under way with expected results in virus phylogeny and evaluation on similarity with recent circulating strains in United States, Canada and European countries.

Conclusions: EV-D68 was detected in a high positive rate (69%) among EV positive cases. This positive rate of EV-D68 was higher compared to the positivity rate of 10.2%, calculated in a European study during 2014 [2]. This finding could be linked to the selection of severe and hospitalized patients in present study, highlighting the involvement of EV-D68 with severe respiratory disease in children. The identification of EV-D68 is also crucial in respiratory samples in children with clinical diagnosis of neurological illness. This study is the first attempt to describe the prevalence of EV-D68 in severe paediatric cases, in Portugal. The strength of EV-D68 surveillance in paediatric and adult population at the national level will be important to understand the epidemiology of EV-D68, age-related susceptibility and association with disease severity.

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Evaluation of TTV load kinetics among kidney transplant recipients in the first year post-transplant period

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Introduction: Torque teno virus (TTV) is highly prevalent in humans (90%) with a persistent low-level viremia in the immunocompetent host. Patients who undergo kidney transplant have a high risk of blood-borne viral infections, including the TTV. The objectives of this study are: (i) to assess the level and kinetics of TTV DNA in patients after kidney transplantation; (ii) to investigate the possible association with different conditioning regimens and the kinetics of TTV DNA load; and (iii) to correlate the TTV DNA level with the post-transplant immune reconstitution.

Material and methods: TTVDNA load was assessed in a series of blood samples collected at 15, 30, 60, 90, 180, and 360 days after transplant from 78 kidney transplant recipients (KTRs) prospectively monitored for opportunistic virus infections at the Molecular Virology Unit, Fondazione IRCCS Policlinico San Matteo. The total T-cell, T-CD4$^+$, and T-CD8$^+$ lymphocyte counts were retrospectively retrieved at the same time points used for the TTV DNA load analysis.

Results: In 72/78 (92.3%) patients, TTV DNA was detected at 15 days after transplantation. At 60 days after transplantation, all patients were positive for TTV infection. In 29/78 (37.2%) patients, the peak of viral load was reached at 180 days after transplantation, in 24/78 (30.8%) at 360 days and in 20/78 (25.6%) at 90 days. Only 4 (5.1%) and 1 (1.3%) patients reached the peak of viral load at 60 and 15 days after transplantation, respectively. A significant increase of TTV DNA load was observed between 15 days (median