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Abstract (poster session)

Development of a new method for BK polyomavirus genotyping: application to transplant recipients

S. Burrel, M. Gits-Muselli, Z. Aït-Arkoub, S. Nguyen, N. Arzouk, H. Agut, D. Boutolleau* (Paris, FR)

Objectives: In immunocompromised patients, BK virus (BKV) reactivation may result in nephropathy in kidney transplant (KT) recipients or haemorrhagic cystitis in haematopoietic stem cell transplant (HSCT) recipients. BKV strains are classified into four 4 different genotypes (I to IV). BKV genotyping has been historically based on the analysis a 327-bp variable region of the gene coding for the major capsid protein VP1. The aim of this work was to develop a new method for BKV genotyping based on the sequencing of the full-length gene coding for VP1 and to determine BKV genotypes among KT and HSCT recipients. Methods: Primers were designed to amplify, by nested PCR, and to sequence the full-length VP1 coding region (1,089 bp). Amplified products were analyzed with the automated sequencer ABI 3100 Genetic Analyzer (Applied Biosystems). This new method was applied to 52 EDTA whole blood specimens obtained from 40 KT and 12 HSCT recipients (34 men, 18 women, median age: 48 years) experiencing BKV active infection (median BKV load in blood: 5.5 log). All nucleotide and amino acid VP1 sequences were aligned with SeqScape v2.5 software using BKV Dunlop strain as a reference (GenBank accession number V01108). A phylogenetic tree was constructed by the neighbor-joining method using ClustalW program, including the VP1 sequences from the different reference BKV strains representing the main genotypes obtained from GenBank. Results: The sensitivity of the VP1 nested PCR was 500 copies/mL. At the nucleotide level, the interstrain identity of VP1 gene ranged from 91.9 to 99.2%. At the amino acid level, a total of 45 amino acid changes were identified, that is 12.4% of the total codons of the protein. Each strain harboured a mean number of 8.8 amino acid changes. Seven amino acid changes were evidenced among at least 50% of the BKV strains. As a whole, the distribution of BKV genotypes among transplant recipients was as follows: I (81%), II (8%), III (0%), IV (12%). Among genotype I, BKV Ia, Ib1, Ib2, and Ic subgroups represented 5%, 31%, 64%, and 0%, respectively. Only BKV subgroups Ib1 and Ib2 were identified among HSCT recipients. Conclusion: We report here the development of a new method for the sequencing of the full-length VP1 coding region allowing BKV genotyping. Our results evidenced the high variability of VP1. This method constitutes a useful tool for further studies on BKV pathogenicity according to the genotypes.