1. Introduction

The Polyomaviridae family includes the human polyomaviruses BK (BKV), JC (JCV), and simian virus 40 (SV40) (1,2), which exhibit a genome identity of approx 69%, with the lowest similarity in the regulatory region (transcription control region) (3). Polyomavirus infections are typically acquired early in the childhood. Both BKV and JCV are ubiquitous and more than 80% of adults are seropositive. SV40 naturally infects the rhesus macaque and was inadvertently introduced as a pathogen into the human population as a contaminant of early polio vaccines. It has been estimated that up to 30 million people in the United States were exposed to live SV40 between 1955 and 1963 (3). Based on serological studies, the prevalence of SV40 infection in humans ranges from 2% to 20% (3,4), with values increasing with age (5). After a primary infection, these viruses are maintained by the host in a persistent state in kidney (6,7) and possibly lymphocytes. BKV, JCV, and SV40 have been evidenced in peripheral blood lymphocytes of normal and immunocompromised individuals (8–13). Although primary infection is usually asymptomatic, clinical manifestations are observed in immunocompromised individuals, including AIDS patients and transplant recipients (14–16). Given the increasing incidence of polyomavirus-associated pathology, clinical management and possibly prevention could benefit from advancements in diagnosis and monitoring of infections.

Several studies reported a correlation between BKV and interstitial nephritis in renal transplant...
recipients, in which the degree of immunosuppression is thought to allow or induce reactivation of the virus \((15,17–19)\). The specific risk factors for BKV-associated nephropathy (PVAN) are unknown; however, it seems to develop in the context of deep immunosuppression, particularly with triple immunosuppressive therapy, comprising tacrolimus or mycophenolate mofetil and corticosteroids \((15,20)\). Based on serological studies, it has been reported that up to 45% of kidney transplant patients experiences BKV reactivation \((21)\) and this may cause nephropathy and renal failure in as many as 8% of subjects, eventually leading to the graft loss \((22)\). Clinical management of PVAN includes lowering or switching of the immunosuppressive regimen, the success of which depends on early recognition. More recently, antiviral drugs cidofovir and leflunomide have been used in these cases. Moreover, BKV is frequently detected in urine specimens from bone marrow transplant recipients with hemorrhagic cystitis, the etiopathogenesis of which has been hypothesized to be related to viral reactivation \((23)\).

JCV has been associated with progressive multifocal leukoencephalopathy (PML). This demyelinating disease is characterized by lesions in the white matter of the cortex, which represent areas of viral replication within oligodendrocytes, the myelin-producing cells of the central nervous system \((24,25)\). During active viral replication, lytically infected oligodendrocytes succumb. The clinical course of the disease is progressive and the prognosis remains poor \((26)\). Although PML was once considered rare, in recent years, since the AIDS epidemic \((24,27)\) and the increase of solid organ and bone marrow transplantations \((28–33)\), it has become an increasingly common neurological disease.

Recent molecular studies have evidenced an association between SV40 and some human neoplasms. SV40 DNA sequences have been detected in primary brain and bone tumors, pleural mesotheliomas, and non-Hodgkin’s lymphomas \((34–36)\), the same types of malignancies induced by SV40 infection in the hamster model \((3,37)\). A recent study has evidenced the risk of false-positive results as a result of contamination by common laboratory plasmids containing SV40 sequences \((38)\). SV40 DNA has also been detected in renal biopsies obtained from pediatric kidney allograft recipients \((5)\).

In addition to medical relevance of advancements in diagnosing and monitoring polyomavirus infections, an improved understanding of the virus–host relationship could contribute to develop management strategies for polyomavirus-associated diseases. In recent years molecular biology techniques, including conventional polymerase chain reaction (PCR), have been used to detect and identify polyomaviruses; however, such techniques show some drawbacks, such as the potential for false negatives because of low detection sensitivity. The introduction of new molecular methods has led to significant advances in research and diagnostics. Epidemiological and pathogenic studies on polyomavirus infections could benefit from multiplex PCR methods \((39)\). A sensitive multiplex nested PCR (mmPCR) method was developed for simultaneous detection and typing of polyomaviruses BKV, JCV, and SV40, with the aim of simplifying detection and reducing time and costs. In the first amplification reaction, the pair of primers was used to amplify a sequence of either BKV, JCV, or SV40 at a highly conserved genomic region (large T antigen gene). In the second amplification specific primers were used for each of the three polyomaviruses. Accuracy, reproducibility, specificity, and sensitivity of these assays were established.

2. Materials and Methods

2.1. JCV, BKV, and SV40 DNA

To determine the sensitivity of the PCR assay, plasmids pJCV4-1, pBKV33-1, and pSV40 (courtesy of T. Musso e D. Lembo), containing JC, BK, and SV40 virus DNA, respectively, were linearized and a stock preparation of each at 1 × 10^6 copies/mL was diluted to 1 copy/mL by a series of 10-fold dilutions.

2.2. Clinical Specimens and DNA Extraction

Renal biopsies from 29 transplant recipients (22 men, 7 women; mean age 58.9 ± 9.3 years) who underwent kidney transplantation at Renal Transplant Unit of the S. Giovanni Battista