

Clinical Role of Quantitative Viral Load Monitoring for Adenovirus in Immunocompromised Patients

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Adenoviruses (AdVs) are increasingly recognized as viral pathogens that can cause significant morbidity and mortality, especially in immunocompromised patients. In the immunocompromised transplant recipient, AdVs fatality rates have been reported to be as high as 60% for patients with pneumonia and 50% for patients with hepatitis, and 25% overall. Thus, management of serious adenovirus infections requires early diagnosis, differentiation from other conditions, and the ability to monitor the course of the infection after treatment. Although several antiviral therapies have been reported for the treatment of AdV infections, none have been documented to have definite clinical benefit. However, it appears that any antiviral therapy, if contemplated, must be initiated in the earliest stages of disease to be effective and must be used carefully because of their potential for toxicity. This means timely diagnosis of AdV infection and the ability to monitor viral load during treatment is essential.

Recently, quantification of viral DNA load has been reported to be a valuable tool to monitor various infections. Use of real-time, quantitative, Polymerase Chain Reaction (RQ-PCR) technology allows accurate early detection and follow-up to detect possible progression or dissemination of the disease, as well as monitoring of the therapeutic response.

However, unlike many other viral pathogens that have a single strain, the adenoviridae family has more than 50 different strains. They vary in host specificity, disease type, and genomic contents. Hence, PCR assays employing a single set of primers and probe to detect all the strains could be a major challenge.

We have developed and employed a real time PCR assays for monitoring AdV infection in the clinical scenario. This assay allows rapid turnaround time (same day in many cases), coupled with high sensitivity, specificity, reproducibility and accuracy. Here we report the clinical role of monitoring AdV infection using our assay.

Methods: We utilized the real time PCR technique (TaqMan) to quantify AdV DNA in clinical specimens using primers designed for a region of the hexon gene that is conserved among all AdV serotypes. Additionally, to monitor the PCR product during amplification, we designed a minor groove-binding (MGB) label to allow the use of a small oligonucleotide probe to detect all AdV serotypes.

Results: The assay detected 14 of 14 different AdV serotypes (1, 2, 3, 4, 5, 6, 7, 8, 11, 19, 31, 34, 37, and 42) obtained from ATCC. We screened 154 clinical specimens and identified 5 positive patients. The viral load range was 1.5×10^2 – 3.7×10^5 copies/ml for blood, 1.0×10^2 – 2.2×10^6 copies/ml for urine and 3×10^1 – 7.4×10^3 copies/cell, respectively.

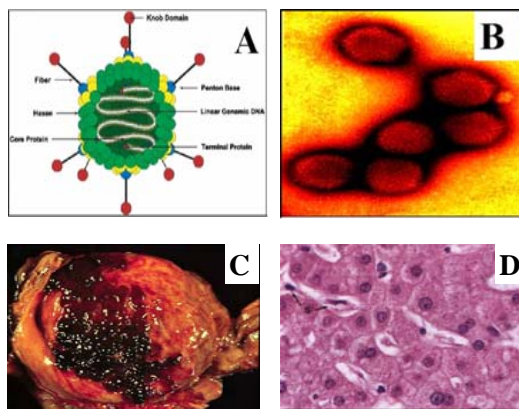


Figure 1: Human adenoviruses are DNA viruses with an outer protein coat, containing 152 subunits of capsomeres (A). These capsomeres are arranged in an icosahedral structure with 20 sides and 12 vertices. The capsid subunits are of three morphologic types: hexons, pentons and fibers. Adenovirus as visualized by electron microscopy (B) is 70-90nm diameter and can cause number of clinical symptoms, of which hemorrhagic cystitis (C) and hepatitis (D) are commonly seen in BMT patients.

We studied 4 AdV-positive patients serially which are presented in more detail. Pt #1, with acute GVHD, following bone marrow transplantation presented with fever, diarrhea and progressive liver dysfunction. The blood PCR for AdV was positive and the viral load increased as the liver function worsened from 5,750 copies/ml with AST of 81 to $11,370$ copies/ml with AST of 198 and progressing to 1.9×10^6 copies/ml with AST of 1061). The patient expired following the peak AdV load of 1.4×10^8 copies/ml. The patient's liver biopsy by EM was found to have particles suggestive of AdV. Pt #2, with chronic GVHD, on immunosuppressive therapy presented with an acute febrile illness and was found to have an initial blood AdV load of 50,000 copies/ml. He promptly improved after treatment with high dose IV IgG, with the viral load dropping to 200 copies/ml. A few weeks later, after a 2nd increase in viral load (6,200 copies/ml), he was again treated with IV IgG. This was again followed by a decrease in viral load to 200 copies/ml. Four weeks later, after another dose of IV IgG, the viral load became undetectable. The other patients also had clinical correlation with viral load.

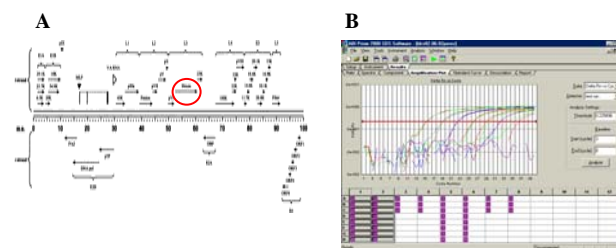


Figure 2: Adenoviruses are double-stranded DNA viruses with genomic size of 30-42kbp long, depending on serotype (A). The genomic structure shows the order and direction of gene expression from the early to the late genes. To detect adenovirus, we designed primers and probe based on the conserved hexon region. The red circle indicates the location of hexon gene, our detection target for real time quantitative PCR. Above is a screen snap shot of real time fluorescence curves on an ABI SDS 7000 machine for AdV (B). The curves on the left represent amplification of higher copy numbers whereas curves on the right represent lower copy numbers.

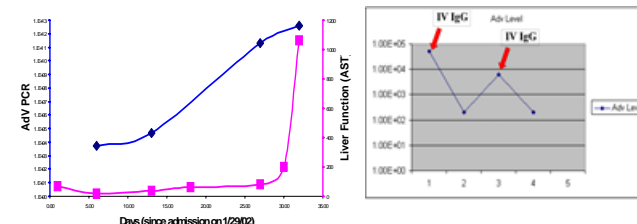


Figure 3: Clinical utility of real time quantitative PCR for detecting and monitoring AdV infection in bone marrow transplant. (Left) In patient 1, the progression of AdV viremia by PCR correlated with worsening liver function tests. AdV load increased along with the AST levels. The viral load is shown in logarithmic scale. (Right) In patient 2, viral load correlated with the patient's clinical condition and helped monitor therapy. AdV load increased with worsening symptoms and decreased following administration of IV IgG.

Conclusions: The real time PCR assay for AdV can provide a specific and rapid diagnosis of life-threatening AdV infection in immunocompromised patients and would be useful in guiding as well as monitoring the response to specific therapy. The use of an MGB probe allowed the detection of multiple AdV serotypes with limited sequence homology.

