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### Case Report

# Detection of Human Adenovirus (species-C, -D and -F) in an allogeneic stem cell transplantation recipient: a case report



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Human Adenovirus (HAdV) commonly causes mild clinical symptoms in immunocompetent patients. In immunocompromised individuals, such as patients submitted to allogeneic stem cell transplantation (aSCT), HAdV infection can cause prolonged and disseminated disease, resulting in a worse prognosis for the patient or even death.<sup>1</sup>

A 57-year-old Brazilian man was diagnosed with chronic myeloid leukemia and during treatment with imatinib he developed resistance secondary to a T315I mutation in the BCR-ABL kinase domain. A human leukocyte antigen (HLA)-identical sibling donor was available, and the patient was submitted to stem cell transplantation in October 2012 with non-myeloablative conditioning based on fludarabine (150 mg/m<sup>2</sup>) and busulfan (16 mg/kg). Trimethoprim/sulfamethoxazole, acyclovir and fluconazole were given as antimicrobial prophylaxis. Graft-versus-host disease (GVHD) prophylaxis was achieved with cyclosporine (3 mg/kg) from Day 1 prior to transplant (D - 1) and a short course of methotrexate (15 mg/m<sup>2</sup> on D + 1 and 10 mg/m<sup>2</sup> on D + 3, D + 6, D + 11 post-transplant).

Cell source was non-stimulated bone marrow and  $2.8 \times 10^8$ /kg of total nucleated cells without ABO incompatibility were infused.

This study was approved by the Research Ethics Committee of the Hospital Araújo Jorge/Associação de Combate ao Câncer em Goiás (ACCG: protocol #108.396). The patient signed a consent form for his clinical samples (feces and sera) to be monitored for gastroenteric viruses. The first sample was collected on D + 1, and subsequently samples were obtained weekly until patient discharge. Samples were then collected during outpatient visits. Samples were processed using commercial kits (QIAamp Stool Mini Kit and QIAamp MinElute Spin Kit, QIAGEN, Freiburg, Germany for stool and serum, respectively), following the manufacturer's instructions.

To screen for HAdV, samples were subjected to quantitative polymerase chain reaction (PCR - Taqman) as previously described with adaptations.<sup>2,3</sup> Briefly, pure and 1:10 diluted DNA was added to a 25  $\mu$ L mix containing 1 $\times$  of Master Mix (applied Biosystems), 0.9  $\mu$ M of each primer and 0.225  $\mu$ M of

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**Table 1 – Viral load of HAdV-positive samples and associated clinical symptoms.**

| Clinical sample | Days after transplant | qPCR (copies/mL)   | Species | Clinical symptoms                                            |
|-----------------|-----------------------|--------------------|---------|--------------------------------------------------------------|
| Serum           | D + 9                 | $2.07 \times 10^6$ | F       | Vomit, abdominal pain, mucositis, diarrhea, NoV <sup>+</sup> |
| Stool           | D + 17                | $1.97 \times 10^8$ | D       | Vomit, diarrhea, NoV <sup>+</sup>                            |
| Serum           | D + 76                | $3.78 \times 10^4$ | F       | Cutaneous rash                                               |
| Serum           | D + 153               | $5.79 \times 10^4$ | C       | Diarrhea                                                     |

qPCR: quantitative polymerase chain reaction; NoV: norovirus.

Grade II graft-versus-host disease of the skin and liver was present from D + 41 to D + 76.

Taqman probes labeled with FAM-TAMRA, targeting a region of 72 base pairs. The cycling program was the following: 50 °C for 1 min, 95 °C for 10 min, followed by 45 cycles of 95 °C for 15 s and 60 °C for 1 min. Samples were run with standard curves ( $R > 0.98$ ) constructed using serial dilutions ( $10^{-2}$  to  $10^8$ ) of the pBR322 plasmid containing the HAdV hexon gene. Results are expressed as genomic copies per milliliter (copies/mL).

HAdV-positive samples were also subjected to genomic sequencing in an automatic sequencer (DNA ABI PRISM 3130, Applied Biosystems), using purified nested-PCR products, in duplicates, amplified by primers targeting a conserved region of the hexon gene, as described by Puig et al.<sup>4</sup>

The samples from days D + 1, D + 3 and D + 6 were negative, and the patient remained asymptomatic until D + 9, when he presented nausea, vomit, abdominal pain, fever and diarrhea. At this time, the patient also presented severe leucopenia ( $< 1000$  cells/mm<sup>3</sup>) and lymphopenia ( $< 300$  cells/mm<sup>3</sup>). HAdV species F was detected in serum (GenBank accession number KP894106) with a viral load of  $2.07 \times 10^6$  copies/mL (Table 1). This sample was also positive for norovirus GI.3 identified by reverse transcription PCR.<sup>5</sup> The patient still presented with diarrhea, leucopenia and lymphopenia on D + 17, and his fecal sample tested positive for HAdV species D (GenBank accession number KP894104) with a viral load of  $1.97 \times 10^8$  copies/mL. This sample was also positive for norovirus GI.3.

On D + 21, the patient was discharged. A fecal sample obtained during an outpatient visit on D + 27 was positive for HAdV ( $6.94 \times 10^{10}$  copies/mL) and for norovirus (GI.3); at the time the patient presented with diarrhea, abdominal pain and vomit. The patient returned on D + 41 with a skin rash, vomit, fever and abdominal pain; a clinical examination revealed an acute skin rash ( $< 25\%$  of body surface area) and hyperbilirubinemia (3.14 mg/dL) compatible with acute grade II GVHD. After readmission, the patient was submitted to intravenous rehydration and immunosuppression with cyclosporine (3 mg/kg) and metilprednisolone (2 mg/kg). After five days, the patient's clinical status had improved and the symptoms ceased. He was discharged with a prescription of oral cyclosporine (10 mg/kg) and prednisone (1 mg/kg). At this time, samples were negative for HAdV, but positive for norovirus GI.3.

On D + 76, the patient still presented with acute GVHD, and showed positivity for HAdV species F ( $3.78 \times 10^4$  copies/mL) in a serum sample. Phylogenetic analysis revealed that the HAdV sequence was identical to the sequence found in the sample obtained on D + 9. Progressive reduction of prednisone was made and interrupted on D + 108. Cyclosporine was reduced and removed on D + 180.

On D + 153, the patient was suffering from diarrhea and presented positivity for HAdV species C in serum (GenBank accession number KP894102;  $5.79 \times 10^4$  copies/mL). Subsequent samples were negative for HAdV and norovirus, and the patient remained asymptomatic and without signs of GVHD. On D + 180 after aSCT he was in complete remission and a study of short tandem repeats (STR) showed chimerism compatible with 100% of donor cells and quantitative BCR-ABL transcript negative in peripheral blood. More than three years after transplant, the patient remains alive without immunosuppression.

## Discussion

HAdV infection may result in a worse prognosis for patients submitted to aSCT.<sup>6</sup> In some cases, HAdV infection is clinically diagnosed as GVHD, and the use of immunosuppressive therapy in these cases could further impair the patient's clinical condition.<sup>7</sup> In this case, three different species of HAdV were identified in samples from the same patient up to D + 153.

During the period in which the patient was positive for HAdV species F and D, even though he presented symptoms that are characteristic of enteric HAdV infection (diarrhea, abdominal pain and vomit), he was also positive for norovirus, which may have influenced and perhaps intensified the symptoms.

After a sequence of negative sera samples, one sample was positive for HAdV species F on D + 76 that had an identical sequence to the sample detected on D + 9, this time with lower viral load, suggesting that viremia was intermittent. At this time, we were unable to differentiate viral reactivation from persistent acute GVHD. We hypothesize that the immunosuppressive therapy impaired viral clearance due to the early transplantation phase associated with suppositional delay in T-cell immune reconstitution. This could be owing to inefficient thymopoiesis, probably caused by the intensive conditioning regimen, acute GVHD, use of corticosteroids and the recipient's age.

Although HAdV species F may cause gastroenteritis, the virus was not detected in fecal samples of this patient, nor did the patient present gastroenteric symptoms when HAdV was again detected in serum. HAdV species D, detected on D + 17, has been associated with gastroenteritis, and may have induced the symptoms during the time that fecal samples remained positive. HAdV species C, detected on D + 153, can cause not only diarrhea but also respiratory and urinary symptoms; the latter symptoms were not observed in this case. The asymptomatic HAdV infections have been observed in

transplanted patients with high viral loads, indicating that infection does not always necessarily cause symptoms.<sup>8</sup>

This case report shows that distinct HAdV species may be present in the nosocomial environment, and despite having strict infection and transmission control measures, they are not always sufficient to avoid viral circulation in the hospital, as previously observed.<sup>9</sup>

This patient was the first in a series of patients monitored for HAdV infection (unpublished data). In the patients that followed, HAdV species F and C, with identical genomic sequences as found in the samples of this patient, were also detected. Therefore, we speculate that he was either the first patient to become HAdV-positive in a series of cases, or that he may have been the original carrier who introduced these viruses into the hospital, which remained circulating for at least two more years (data unpublished). It is also possible that HAdV-positivity is a result of adenovirus reactivation from a previously latent infection.<sup>10</sup>

The results highlight that HAdV can be present in the nosocomial environment, with the possibility of it remaining infectious for months after its first introduction, and that existing infection prevention protocols are not sufficient to prevent virus circulation. In Brazil, patients undergoing aSCT are not monitored for HAdV infection, but our data demonstrate the importance of monitoring clinical samples of these patients in order to provide an appropriate treatment when the infection and the clinical symptoms are present.

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### Conflicts of interest

The authors declare no conflicts of interest.

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