Disseminated Adenovirus Infection in Cancer Patients Presenting with Focal Pulmonary Consolidation

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We report disseminated adenovirus (ADV) infection in four adult cancer patients presenting with focal pulmonary consolidation. In all cases, ADV was recovered from respiratory specimens and ADV viremia ($>1 \times 10^5$ copies/ml) was determined by a quantitative PCR assay. Despite antiviral therapy, 3 (75%) patients died. ADV should be considered as a possible cause of severe pneumonia in immunosuppressed patients.

CASE REPORTS

We describe 4 cases of severe adenovirus (ADV) pneumonia in adult cancer patients at Memorial Sloan-Kettering Cancer Center (MSKCC) (Table 1).

Case A. A 73-year-old man with chronic lymphocytic leukemia (CLL) presented with fever, myalgia, and malaise for 3 days. His most recent chemotherapy consisted of rituximab and cyclophosphamide, given 14 months earlier. One week prior to presentation, he was treated for dermatomal herpes zoster as an outpatient. On admission, a chest X-ray (CXR) showed focal left retrocardiac opacity (Fig. 1A). Levofloxacin was given for presumed community-acquired pneumonia (CAP). On hospital day 4, he developed hypoxia and had persistent fever and productive cough. Computerized tomography (CT) of the chest showed new consolidation in the left lower lobe and posterior lingula. On day 7, bronchoscopy showed acute mucosal inflammation. ADV serotype 3 was isolated from a bronchoalveolar lavage (BAL) fluid viral culture. The ADV load in the blood determined by PCR was 3.66×10^5 copies/ml. He received one dose of intravenous (i.v.) cidofovir and one dose of i.v. immunoglobulin (IgG), followed by 3 doses of oral CMX001 (200 mg) given weekly through Emergency Investigational New Drug (EIND) provisions. The patient's condition improved, and he was discharged to his home after 18 days of hospitalization.

Case B. A 34-year-old woman with a recent diagnosis of breast cancer presented to a hospital other than MSKCC 4 days after her second cycle of chemotherapy with cyclophosphamide and doxorubicin with fever and chills for 4 days. Additionally, she had recently had bilateral conjunctivitis treated with moxifloxacin eye drops. She was prescribed oral levofloxacin and was discharged to her home but the next day presented to MSKCC with persistent fever up to 39.4°C , malaise, and nonproductive cough. She reported that her children had had a recent viral infection. Chest CT on admission revealed a left lower lobe consolidation (Fig. 1B). Vancomycin and levofloxacin were given i.v. A throat culture was negative for bacteria and viruses. On hospital day 4, she developed hypotension and confusion and was transferred to the intensive care unit (ICU) and intubated. On day 10, viral culture of the BAL fluid tested positive for ADV. Amplification and sequencing of hypervariable regions 1 to 6 of the hexon gene allowed the identification of the virus as ADV type 7 (1). The ADV load in the blood

determined by PCR was 3.9×10^7 copies/ml. The patient received one i.v. dose of cidofovir. However, she expired on day 15 from multiorgan system failure (MOSF).

Case C. A 75-year-old woman with non-Hodgkin's lymphoma for 15 years treated with multiple regimens (most recently with rituximab and cyclophosphamide until 6 months prior to admission) initially presented to her primary care physician (PCP) with a fever of 38°C , productive cough, sore throat, runny nose, fatigue, and decreased appetite for 3 days. She received oral azithromycin without improvement. On admission, physical examination was unremarkable except for blood pressure (89/59 mm Hg). A nasopharyngeal (NP) swab was negative for respiratory viruses by a FilmArray Respiratory Panel (RP) (BioFire, Inc., Salt Lake City, UT). CXR showed right upper lobe consolidation (Fig. 1C). Vancomycin, cefepime, and azithromycin were administered i.v. On hospital day 3, she developed respiratory failure, transaminitis, and acute renal failure and was transferred to the ICU. Bronchoscopy was performed on day 4. A BAL fluid viral culture tested positive for ADV. Amplification and sequencing of hypervariable regions 1 to 6 of the hexon gene allowed the identification of the virus as ADV type 4. The ADV load in the blood determined by PCR was 9.7×10^9 copies/ml. She received one i.v. dose of cidofovir with i.v. IgG. She expired on day 6 from MOSF.

Case D. A 43-year-old man with recently diagnosed diffuse large B-cell lymphoma, treated with two cycles of rituximab, cyclophosphamide, doxorubicin, and prednisolone 2 months prior to the present illness, presented to his PCP complaining of intermittent dry cough for 6 days with productive sputum for 1 day. Oral levofloxacin was prescribed. The following day, he presented to MSKCC with fever. Physical examination results were unremarkable. The absolute neutrophil count was $600/\mu\text{l}$. CXR re-

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FIG 1 Radiographic findings of ADV cases, showing focal consolidation. (Cases A and B) Computerized tomography (CT) of the chest on admission day +3 and on admission, respectively. (Cases C and D) Posteroanterior chest X-ray (CXR) on admission. Descriptions of radiologic findings are as follows. Chest CT of case A shows left lower lobe and posterior lingula consolidation. Chest CT of case B shows left lower lobe consolidation. CXR of case C shows right upper lobe consolidation. CXR of case D shows left lower lobe consolidation.

vealed left lower lobe consolidation (Fig. 1D). An NP swab was positive for ADV by FilmArray RP. Liver function tests (LFT) were within the normal range. Piperacillin-tazobactam and azithromycin were given for pneumonia in the setting of neutropenia. On hospital day 4, he developed diarrhea. *Clostridium difficile* PCR was negative. He continued to have intermittent high fevers with rigors. Stool viral culture was positive for ADV. On day 5, he was noted to have increased LFT ($>7\times$ upper limit of normal). On day 6, he was transferred to the ICU for progressive confusion and MOSF. The ADV load in the blood determined by PCR was 1.6×10^7 copies/ml. He received i.v. cidofovir with i.v. IgG. He expired on day 8 from MOSF. Amplification and sequencing of hypervariable regions 1 to 6 of the hexon identified an isolate from the stool culture as ADV type 4.

ADV is the cause of up to 3% of community-acquired pneumonia (CAP) (2) and has been implicated in sporadic cases and outbreaks of severe pneumonia in healthy adults. ADV is also a known cause of fatal pneumonia in stem cell transplant (SCT) recipients. In contrast, only a few cases have been reported in non-SCT adult cancer patients (3, 4).

We report disseminated ADV infection in four non-SCT cancer patients. All patients presented with acute respiratory illness and focal pulmonary consolidation. On the basis of symptoms and initial radiologic findings, all patients were initially treated with antibiotics for CAP. The time from admission to diagnosis of ADV pneumonia was a median of 8.5 days (range, 6 to 12). Bronchoscopy was performed in three cases due to progression of

TABLE 1 Characteristics, treatments, and outcomes of ADV pneumonia cases^a

Case	Age/sex	Primary diagnosis	Last cancer treatment, day ^b	ALC	Sites positive for ADV/day	Peak ADV load in blood/day	ADV type ^c /site	AST/ALT on admission (AST/ALT at peak)	Antiviral treatment	Treatment start day	Cause of death/day
A	75/M	Chronic lymphocytic leukemia	Rituxinab, cyclophosphamide, –395 days	4,900	NP Cx/D +6; BAL fluid Cx/D +7; stool Cx/D +11	4.9×10^5 /D +9	3/BAL fluid	24/30 (115/113)	Cidofovir (1 mg/kg of body wt i.v. $\times$ 1 dose), CMX001 (200 mg $\times$ 3 doses)	D +9	N/A
B	34/F	Breast cancer	Doxorubicin, cyclophosphamide, –7 days	140	NP Cx/D +1; BAL fluid Cx/D +5; stool Cx/ND	3.9×10^7 /D +9	7/BAL fluid	39/35 (415/ND)	Cidofovir (1 mg/kg i.v. $\times$ 1 dose)	D +11	Respiratory failure, MOSF/D +15
C	75/F	Non-Hodgkin's lymphoma, leukemic phase	Rituxinab, cyclophosphamide, –180 days	4,400	NP PCR/Neg; BAL fluid Cx/D +7; stool Cx/ND	9.7×10^9 /D +4	4/BAL fluid	63/210 (428/7,275)	Cidofovir (3 mg/kg i.v. $\times$ 1 dose)	D +5	Respiratory failure, MOSF/D +7
D	43/M	Diffuse large B-cell lymphoma	Rituxinab, cyclophosphamide, doxorubicin, prednisone, –6 days	64	NP PCR/D 0; BAL fluid Cx/ND; stool Cx/D +5	1.2×10^9 /D +3	4/Stool, NP	29/46 (3,780/12,330)	Cidofovir (3 mg/kg i.v. $\times$ 2 doses), ribavirin (6 g inhalation $\times$ 1 dose)	D +6	Respiratory failure, MOSF/D +8

^a M, male; F, female; ADV, adenovirus; D, day; Neg, negative; ALC, absolute lymphocyte count/mm³ on admission; BAL, bronchoalveolar lavage; NP, nasopharyngeal swab; ND, not done; ALT, alanine aminotransferase; AST, aspartate aminotransferase; i.v., intravenous; N/A, not applicable; MOSF, multisystem organ failure; Cx, viral culture.

^b Numbers of days shown are relative to the day of admission for ADV pneumonia (admission day = day 0).

^c Quantitative real-time ADV PCR in whole blood was performed by Focus (Cypress, CA, USA) for case B or Viracor-IBT Laboratories (Lee's Summit, MO, USA) for cases A, C, and D. Values are copies/ml.

^d ADV typing was determined by a neutralization assay performed by Focus for case A or by dideoxysquence analysis of hypervariable regions 1 to 6 of the hexon gene performed by the Laboratory of Viral Diseases, Wadsworth Center, New York State Department of Health for cases B, C, and D (1).

^e ALT, reference range 5–37 Units/L; AST, reference range 10–37 Units/L.

^f NP PCR for ADV was performed by the FilmArray Respiratory Panel (RP) (BioFire, Inc., Salt Lake City, UT, USA).

symptoms despite antibiotics. Viral cultures of the BAL fluid were all positive for ADV. Further typing of the ADV isolated revealed serotype 3 (case A) and type 4 (cases C and D) and type 7 (case B) (Table 1).

Human adenoviruses are divided into seven species (A to G) and more than 50 recognized serotypes. Species B (in particular, serotypes 3, 7, 11, 14, and 21) and species E (serotype 4) have most often been associated with epidemic acute respiratory illness (5). During the period of interruption of the United States military adenovirus vaccine program (for ADV serotypes 4 and 7) from 1999 to 2010, Potter et al. reported 8 adenovirus-associated deaths in military recruits caused by ADV types 4, 7, and 14. Of the 8 patients, only 3 had adenovirus infection identified before death (6). In a large nationwide epidemiologic study of U.S. military and civilian medical facilities, types 3, 2, and 1 were most prevalent among civilians whereas type 4 accounted for 93% of all ADV types from military trainees. Importantly, more than 50% of ADV isolates recovered from civilians were associated with hospitalization (7). ADV type 3 is one of the most prevalent serotypes detected globally, and an association with more-severe disease has been reported for specific ADV type 3 subgenomes (8).

Limited data exist on ADV disease in cancer patients. Cavalli-Björkman et al. reported fatal ADV hepatitis in a patient with CLL treated with alemtuzumab (3). Hough et al. reported fatal ADV hepatitis in 3 pediatric patients with acute lymphocytic leukemia (9). Zahradnik et al. reported a mortality rate of 60% among 15 immunocompromised patients with ADV infection (4).

Lymphocytes are crucial for the control of viral infections, including ADV. T-cell depletion has been identified as a risk factor for ADV disease. Additional risk factors for ADV viremia in SCT recipients are young age and acute graft-versus-host disease (GVHD) (10). In addition, high doses of sequential chemotherapy may induce severe lymphocyte depletion (11). Of the patients in the present report, 3 had lymphoid malignancies and 1 was being actively treated for breast cancer. Two patients had absolute lymphocyte counts below $150/\text{mm}^3$ on admission. The remaining 2 had elevated lymphocyte counts but could be considered functionally lymphopenic due to underlying disease (CLL and lymphoma) and/or treatment (rituximab).

Because the initial radiographic presentation of ADV pneumonia may be a focal consolidation, timely diagnosis requires a consideration of viral etiologies and specific diagnostic tests for ADV infection (12). As standard practice at MSKCC, patients presenting with upper respiratory symptoms are routinely tested for respiratory viruses. In September 2011, direct fluorescent antibody assay and viral culture of NP swabs for detection of respiratory viruses were replaced by the multiplex PCR assay FilmArray RP version 1.6. Cases A and B presented prior to September 2011; therefore, ADV was detected by viral culture of an NP swab. Cases C and D presented after September 2011, and NP swabs were tested by FilmArray RP version 1.6. ADV was detected in case D (type 4) but not detected in case C (subsequently found to have ADV type 4 by viral culture of stool).

Detection of ADV presents a known challenge for molecular multiplex assays. In the study by Pierce et al., the sensitivity of Filmarray RP version 1.6 was 46% compared to standard individual real-time quantitative PCR assays (13, 14). Among the currently FDA-approved molecular assays that detect more than 3 respiratory pathogens, the sensitivity for detection of ADV ranges from 57.1% to 100%; with a sensitivity of 57.1% reported for

FilmArray RP v1.6 (13, 14). The low sensitivity of FilmArray RP v1.6 for ADV specifically relates to ADV C, serotypes 2 and 6 (13). The newly released FilmArray RP (version 1.7) includes a second ADV assay designed to enhance the sensitivity of the panel. Compared to FilmArray RP v1.6, v1.7 demonstrated superior performance for detection of ADV, with sensitivity ranging from 83% to 91% (15–17). The enhanced performance is likely due to an increased ability to detect a wider range of serotypes with a lower limit of detection (demonstrated specifically for serotypes 4 and 7) (17). Despite improved performance, multiplex assays may miss some cases of ADV-infected patients. Immunocompromised patients at risk for severe infection would benefit from confirmatory testing in the setting of a negative result by a multiplex assay.

All our cases had ADV viremia, with viral loads ranging from 4.9×10^5 to 9.7×10^9 copies/ml; however, blood ADV PCR was ordered after detection of ADV from a respiratory specimen. Rising ADV viremia is a predictor of ADV disease in stem cell transplant (SCT) recipients, and the majority of SCT recipients with ADV disease have viral loads $\geq 1 \times 10^4$ copies/ml (10). The clinical significance of ADV viremia has not been validated in non-SCT cancer patients. We advocate checking ADV in the blood in patients with lymphoid malignancies or profound lymphopenia who present with fever, pneumonia, gastrointestinal (GI) complaints (diarrhea, transaminitis), and/or a history of recent viral syndrome or contact with children with viral syndrome. Among our patients, three had LFT ≤ 1.5 times the upper limit of normal (ULN) on admission and $>7 \times$ ULN by day 9. One patient developed fulminant hepatitis, presumably due to ADV.

Safe and effective therapy for ADV is a major unmet medical need. Among our patients, the only patient who survived was treated with CMX001 under EIND provision. Timely diagnosis with treatment may have contributed to the positive outcome. Brincidofovir (CMX001; Chimerix Inc., Durham, NC) has a favorable safety and tolerability profile and appeared to be a promising candidate for the treatment of ADV infections in a recent exploratory clinical trial and in case reports (18, 19).

In summary, ADV may be underrecognized as a cause of fatal pneumonia and disseminated infection in cancer patients. In our cases, the initial clinical presentation and radiographic findings were consistent with bacterial pneumonia. However, viral etiologies should be considered in patients with severe lymphopenia or lymphocyte dysfunction with history of recent viral syndromes or GI complaints. While rapid molecular diagnostic assays of NP specimens are very helpful in establishing an early diagnosis when positive, a negative test for ADV may not be sufficient to rule out ADV pneumonia. Additional molecular tests for detection of ADV in blood or BAL fluid may be useful for early diagnosis of severe ADV infection in immunocompromised patients.

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