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Case report

A case of severe pneumonia with viremia caused by adenovirus B7 identified by off-label use of a multiplex PCR system

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ABSTRACT

Severe infection with human adenovirus (HAdV) is uncommon in adults, and the lack of reliable point-of-care testing makes the diagnosis challenging. A 39-year-old immunocompetent Indian man developed severe pneumonia, and his condition became life-threatening despite antimicrobial therapy. While sputum and blood cultures remained negative, a multiplex PCR respiratory panel (Filmarray Respiratory Panel), which is only approved for use with nasopharyngeal samples, detected HAdV in the serum and tracheal aspirates on day 5. We therefore initiated ganciclovir, steroids, and intravenous immunoglobulin. The patient's respiratory condition improved significantly, and he eventually recovered without complications. We later confirmed that conventional PCR of serum detected HAdV-B7. Our case illustrated that a respiratory panel using multiplex PCR successfully detected HAdV in unapproved samples. Such off-label analyses may support the early diagnosis of infections caused by pathogens that are difficult to identify by routine microbiological examination.

Introduction

Human adenovirus (HAdV) generally causes mild disease [1,2]; however, HAdV pneumonia has gained attention due to outbreaks and its severe presentation [1,2]. The diagnosis of HAdV pneumonia is challenging due to the absence of specific clinical manifestations [1,3,4]. Cultures and conventional PCR are the standard diagnostic procedures, but both require hours to days to yield results [1,4] and are available only in centralized laboratories.

Point-of-care multiplexed PCR systems have enabled the rapid and accurate identification of pathogens, supporting the diagnosis of respiratory, gastrointestinal, and central nervous system infections [5]. The Filmarray Respiratory Panel (bioMérieux, NC, USA) can detect a number of common respiratory pathogens (eight viruses [adenovirus, coronavirus, human metapneumovirus, human rhinovirus, enterovirus, influenza virus, parainfluenza virus] and three bacterial species [*Bordetella pertussis, Chlamydophila pneumoniae, Mycoplasma pneumoniae*]), and previous reports have demonstrated its excellent diagnostic capabilities [5,6]. Only nasopharyngeal samples are approved for use with this system, however, and there is no concrete evidence regarding the diagnostic utility of off-label analyses with samples from other body sites [7].

Here, we present a case of severe HAdV pneumonia complicated with systemic infection wherein off-label analysis of serum and tracheal aspirate samples using the Filmarray Respiratory Panel led to early diagnosis.

Case report

A 39-year-old immunocompetent Indian man living in Japan was referred to our hospital in April 2019 for a 5-day history of initial fever, vomiting, and generalized pain, followed by diarrhea. The patient traveled to India for 3 days to visit his relatives and returned to Japan 10 days before his admission. His preexisting conditions included untreated hypertension and obesity with a body mass index of 27.3 kg/m^2.

On initial evaluation, he was febrile but his hemodynamic and respiratory functions were stable: body temperature, 39.7 °C; blood pressure, 168/105 mmHg; heart rate, 120 bpm with sinus rhythm; respiratory rate, 24/minute; and oxygen saturation, 95 % (ambient air). Physical examination revealed ocular conjunctival

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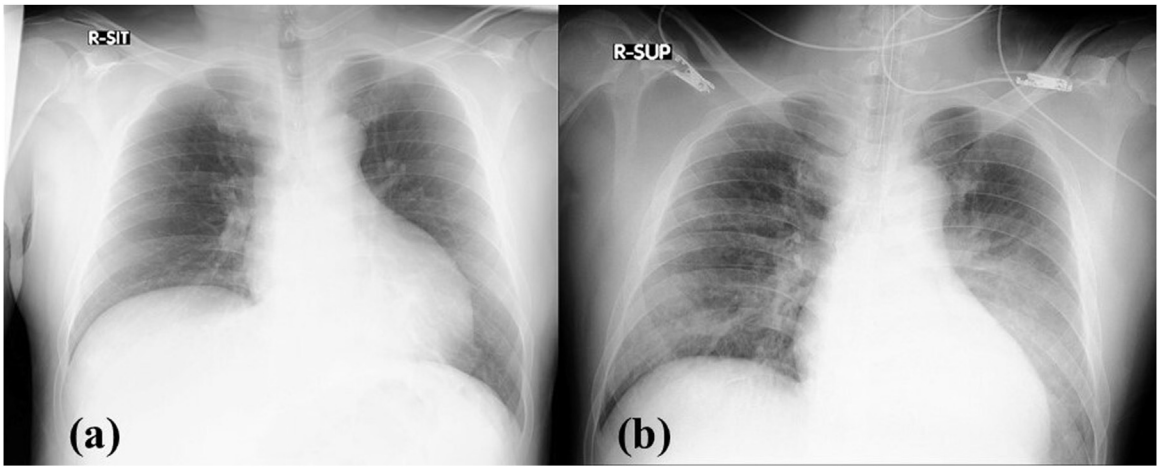

Fig. 1. Chest X-ray images obtained on day 1 (a) and day 5 (b).
A lung consolidation is predominantly left-sided on admission (a), and is bilateral on day 5 (b).

hyperemia and bilateral holo-inspiratory crackles on chest auscultation. Blood tests showed elevated levels of the following: C-reactive protein, 10.12 mg/dL; aspartate transaminase, 63 IU/L; alanine transaminase, 71 IU/L; and lactate dehydrogenase, 509 IU/L. A chest X-ray showed a left lung-dominant consolidation (Fig. 1-a). After two sets of blood and sputum cultures were collected, he was hospitalized and received antimicrobial treatment with ceftriaxone due to the possibility of bacterial pneumonia.

On the day after admission (day 2), his dyspnea worsened and supplemental oxygen therapy was required. We added azithromycin and levofloxacin, and subsequently switched ceftriaxone to meropenem. His respiratory status further declined that evening, and we initiated mechanical ventilation. Computed tomography on the same day showed ground-glass opacities in segments 6, 9, and 10 of the left lower pulmonary region (Fig. 2).

Despite the patient's rapidly worsening respiratory status, his sputum and blood cultures remained negative. His sputum also tested negative for loop-mediated isothermal amplification (LAMP) for *Legionella* spp. To identify the causative pathogens, we used the Filmarray Respiratory Panel version 1.7 to perform molecular examinations of serum and tracheal aspirate samples on day 5. The manufacturer's instructions were followed for all procedures except sample preparation. Specifically, the patient's serum was examined directly, while the tracheal aspirate was diluted in Universal Transport Medium (Copan Diagnostics, CA, USA) due to its viscosity. After that, 300 μL of each sample was loaded into a test kit. Both examinations showed positive results for adenovirus, which led to a tentative diagnosis of pneumonia and viremia caused by adenovirus. Since the manufacturer only supports the use of nasopharyngeal samples for the Filmarray Respiratory Panel, we sent a residual serum sample to a centralized laboratory (LSI, Tokyo, Japan) to validate our results. Eight days later, a qualitative PCR examination performed as previously described [8] identified adenovirus-B type 7 in the sample.

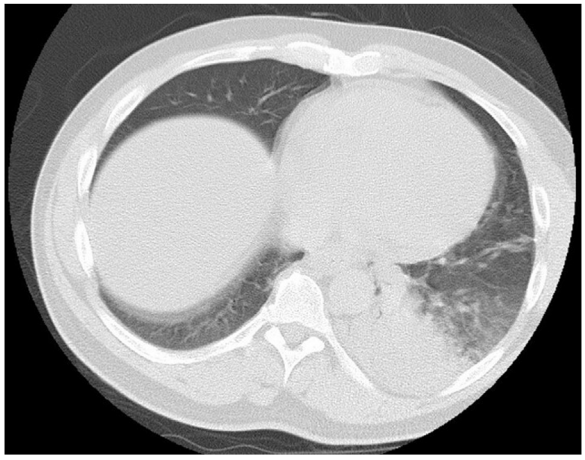

Fig. 2. Computed tomography performed on day 2.
Computed tomography examination shows ground-glass opacities in segments 6, 9, and 10 of the left lower lung.

The patient did not respond to antimicrobial treatment and experienced further respiratory deterioration. On day 5, a chest X-ray demonstrated a bilateral infiltrative shadow (Fig. 1-b) and the patient was diagnosed with acute respiratory distress syndrome. We started combination therapy with ganciclovir, steroids, and intravenous immunoglobulin (IVIG) in addition to antimicrobials. His respiratory status subsequently improved, and the ganciclovir was discontinued in 7 days. Despite the negative sputum culture, we continued to administer antimicrobials for 14 days due to the potential of bacterial coinfection. A significant improvement in the patient's respiratory condition allowed for the withdrawal of mechanical ventilation on day 13. However, flaccid quadriplegia and oculomotor impairment became apparent after extubation. Brain magnetic resonance imaging (MRI) revealed high-signal areas in the bilateral cortex on diffusion imaging. The physical findings and brain MRI results suggested a demyelinating disease of the central nervous system that was most compatible with acute disseminated encephalomyelitis (ADEM) induced by adenovirus infection [9]. We initiated pulse steroid and IVIG therapy, and the patient's palsy gradually improved. He was transferred to another hospital on day 78 for further rehabilitation. By the time of his follow-up visit on day 128, his motor disability had completely resolved without any complications, and he had successfully returned to work.

Discussion

HAdV, a DNA virus, causes upper and lower respiratory tract infections, gastroenteritis, and keratoconjunctivitis [1]. The most severe infections occur predominantly in children, the elderly, and immunocompromised patients [1,2,4]. HAdV pneumonia is considered uncommon in adults, and has been identified in 1.6 % of patients with community-acquired pneumonia (CAP) [10]. However, outbreaks among healthy adults have been reported in recent years, especially in cramped conditions such as military and

healthcare settings [1,2,11]. A previous study reported that the typical clinical characteristics of HAdV pneumonia are high fever, dyspnea, and chest discomfort [11]. Although these signs and symptoms were present in our case, thus far no manifestations specific for HAdV involvement in CAP have been identified [3]. The type of HAdV is associated with the site and severity of infection [1,2], and types 1, 2, 3, 4, 7, 14, 21, and 55 likely cause pneumonia [1,18]. Several studies have suggested that type B7, which was identified in this case, tends to cause severe pneumonia [2], and this type could explain the severity of disease in our patient. A study in the United States showed that compared with non-B7 patients, those with B7 were more frequently hospitalized (69 % *vs*. 84 %, respectively) and admitted to an intensive care unit (18 % *vs*. 39 %, respectively) [2]. The most common HAdV types worldwide were shown to be 1, 2, and 3, with 7 being more common in South Korea, Taiwan, and the United States [1]. In Japan, type 2 was most common (32 %) among lower respiratory infections, followed by 1 (19 %) and 3 (13 %), and type 7 only accounted for 0.4 % [12]. Severe or fatal infections caused by type B7 have rarely been reported in Japan and India; therefore, the travel history of the patient in this case did not provide insight regarding the location of HAdV contraction [12,13].

HAdV infection is commonly diagnosed by enzyme immunoassays, immunofluorescence assays, and PCR [14]. Enzyme immunoassays and immunofluorescence assays provide rapid results but have low sensitivity [14]. Cell culture has high specificity but the results are not available for several days, and it has now been superseded by PCR [14]. Recently, several multiplex PCR devices have been developed and used for point-of-care testing. These assays utilize multiple probes to detect one or more pathogens simultaneously and thereby reduce labor and cost [4,5]. The Filmarray Respiratory Panel demonstrated a sensitivity of 91 % for detecting HAdV [6]. However, HAdV isolated from the upper respiratory tract may not be the causative pathogen [15]; therefore, in cases of non-superficial infections the virus should be identified in samples from normally sterile body sites. In the current case, the Filmarray Respiratory Panel showed positive results for serum and tracheal aspirate samples, neither of which are approved by the manufacturer for this analysis. However, these off-label analyses led to our diagnosis of HAdV pneumonia and viremia, and we validated the serum sample results by conventional PCR. There have been limited data regarding off-label analyses using Filmarray. A previous study showed that the Blood Culture Identification Panel identified causative pathogens with sufficient sensitivity using the following sources: cerebrospinal fluid, ascites, synovial fluid, pleural effusion, respiratory samples, and abscesses [16]. Other studies suggested that the Respiratory Panel could identify pathogens in specimens from the lower respiratory tract [7]. Our case is the first in which the Respiratory Panel was successfully used for off-label analysis to identify HAdV both in serum and tracheal aspirates. Because of their simplicity, quick turnaround time, high diagnostic performance, and ability to detect multiple pathogens, off-label analyses with multiplex PCR may enable the early diagnosis of invasive viral infection, as shown in this case, and improve patient outcomes [7]. Still, we consider it necessary to conduct additional validation studies of these off-label tests.

Although HAdV pneumonia is generally self-limited, cidofovir has been considered the first-choice treatment for severe cases. Cidofovir has more potent *in vitro* activity against HAdV than other agents [1] and is effective for all serotypes [17]. In human subjects, cidofovir improved the survival rate in patients who had undergone allogeneic hematopoietic stem cell transplantation (HSCT) and accelerated viral clearance [17]. However, cidofovir is not available in Japan, and considering the life-threatening condition of our patient, we administered ganciclovir as an alternative [4]. Ganciclovir is mainly used for the treatment or prophylaxis of cytomegalovirus infection and is not approved for HAdV. It exhibits moderate anti-HAdV activity *in vitro*, and its triphosphorylated form inhibits HAdV DNA polymerase [18]. Evidence regarding ganciclovir treatment *in vivo* is scarce. A case report described the successful use of ganciclovir and IVIG in a cardiac transplant patient with severe HAdV pneumonia [19]. In addition, a few retrospective studies demonstrated the potential prophylactic benefit of ganciclovir in HSCT patients [20]. While ganciclovir treatment might have contributed to the improved respiratory condition in our patient, further research is necessary to prove its efficacy.

The limitations of this case are as follows. First, we did not measure the viral load of HAdV. Since it may correlate with the severity of infection [4], a high titer may have been responsible for our patient's unfavorable clinical course. Second, while ADEM was the most likely diagnosis in this case, the diagnostic criteria for ADEM in adults have yet to be established, and there are other possible causes for our patient's flaccid quadriplegia and oculomotor impairment [9].

In conclusion, this is the first reported case in which a multiplex PCR respiratory panel detected HAdV in serum and tracheal aspirates and supported the early diagnosis of HAdV pneumonia with systemic infection. Multiplex PCR using non-approved samples, in addition to careful observation and physical examination, may be helpful because severe systemic infection of HAdV is rarely suspected in healthy adults.

Declaration of Competing Interest

No authors have any conflicts of interest regarding this case.

Sources of funding for your research

No authors have any sources of funding regarding this case.

Consent

Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review process.

Author contribution

A. Sakakura, Y. Akashi, M. Shiigai, H. Suzuki and Y. Hirose contributed in treating the patient and preparing this manuscript. H. Isono supported the preparation of this manuscript.

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References

[1] Lynch JP, Kajon AE. Adenovirus: epidemiology, global spread of novel serotypes, and advances in treatment and prevention. Semin Respir Crit Care Med 2016;37:586–602, doi:http://dx.doi.org/10.1055/s-0036-1584923.
[2] Scott MK, Chommanard C, Lu X, Appelgate D, Grenz L, Schneider E, et al. Human adenovirus associated with severe respiratory infection, Oregon, USA, 2013–2014. Emerg Infect Dis 2016;22:1044–51, doi:http://dx.doi.org/10.3201/eid2206.151898.
[3] Wingfield T, Dearden L, Calvert P, Osanlou O, Johnston B, Chawla A, et al. Adenovirus Type 7 causing severe lower respiratory tract infection in immunocompetent adults: a comparison of two contrasting cases from an intensive care unit in North West England. Clin Infect Pract 2019;2:100007, doi:http://dx.doi.org/10.1016/j.clinpr.2019.100007.

[4] Lion T. Adenovirus infections in immunocompetent and immunocompromised patients. Clin Microbiol Rev 2014;27:441–62, doi: http://dx.doi.org/10.1128/CMR.00116-13.
[5] Ramanan P, Bryson AL, Binnicker MJ, Pritt BS, Patel R. Syndromic panel-based testing in clinical microbiology. Clin Microbiol Rev 2017;31:e00024–17, doi: http://dx.doi.org/10.1128/CMR.00024-17.
[6] Doern CD, Lacey D, Huang R, Haag C. Evaluation and implementation of FilmArray Version 1.7 for improved detection of adenovirus respiratory tract infection. J Clin Microbiol 2013;51:4036–9, doi:http://dx.doi.org/10.1128/JCM.02546-13.
[7] Alby K, Mitchell SL. Lower respiratory multiplex panels for the detection of bacterial and viral infections. Clin Microbiol Newsl 2018;40:131–6, doi:http://dx.doi.org/10.1016/j.clinmicnews.2018.07.005.
[8] Shimada Y, Ariga T, Tagawa Y, Aoki K, Ohno S, Ishiko H. Molecular diagnosis of human adenoviruses d and e by a phylogeny-based classification method using a partial hexon sequence. J Clin Microbiol 2004;42:1577–84, doi:http://dx.doi.org/10.1128/JCM.42.4.1577-1584.2004.
[9] Koelman DLH, Mateen FJ. Acute disseminated encephalomyelitis: current controversies in diagnosis and outcome. J Neurol 2015;262:2013–24, doi: http://dx.doi.org/10.1007/s00415-015-7694-7.
[10] Lieberman D, Shimoni A, Shemer-Avni Y, Keren-Naos A, Shtainberg R, Lieberman D. Respiratory viruses in adults with community-acquired pneumonia. Chest 2010;138:811–6, doi:http://dx.doi.org/10.1378/chest.09-2717.
[11] Park JY, Kim BJ, Lee EJ, Park KS, Park HS, Jung SS, et al. Clinical features and courses of adenovirus pneumonia in healthy young adults during an outbreak among Korean military personnel. PLoS One 2017;12:e0170592, doi:http://dx.doi.org/10.1371/journal.pone.0170592.
[12] National Institute of Infectious Diseases. IASR 2017;38:133–5. https://www.niid.go.jp/niid/en/iasr-vol38-e/865-iasr/7390-449te.html.
[13] Thounaojam AD, Balakrishnan A, Mu AB. Detection and molecular typing of human adenovirus associated with respiratory illness in Kerala. Jpn J Infect Dis 2016;69:500–4, doi:http://dx.doi.org/10.7883/yoken.JJID.2015.414.
[14] Dou Y, Li Y, Ma C, Zhu H, Du J, Liu H, et al. Rapid diagnosis of human adenovirus B, C and E in the respiratory tract using multiplex quantitative polymerase chain reaction. Mol Med Rep 2018;18:2889–97, doi:http://dx.doi.org/10.3892/mmr.2018.9253.
[15] Self WH, Williams DJ, Zhu Y, Ampofo K, Pavia AT, Chappell JD, et al. Respiratory viral detection in children and adults: comparing asymptomatic controls and patients with community-acquired pneumonia. J Infect Dis 2016;213:584–91, doi:http://dx.doi.org/10.1093/infdis/jiv323.
[16] Micó M, Navarro F, de Miniac Daniela, González Y, Brell A, López C, et al. Efficacy of the FilmArray blood culture identification panel for direct molecular diagnosis of infectious diseases from samples other than blood. J Med Microbiol 2015;64:1481–8, doi:http://dx.doi.org/10.1099/jmm.0.000180.
[17] Ison MG. Adenovirus infections in transplant recipients. Clin Infect Dis 2006;43:331–9, doi:http://dx.doi.org/10.1086/505498.
[18] Lenaerts L, Clercq ED, Naesens L. Clinical features and treatment of adenovirus infections. Rev Med Virol 2008;18:357–74, doi:http://dx.doi.org/10.1002/rmv.589.
[19] Duggan JM, Farrehi J, Duderstadt S, Turner NJ, Fekety R. Treatment with ganciclovir of adenovirus pneumonia in a cardiac transplant patient. Am J Med 1997;103:439–40, doi:http://dx.doi.org/10.1016/s0002-9343(97)85997-9.
[20] Lindemans CA, Leen AM, Boelens JJ. How I treat adenovirus in hematopoietic stem cell transplant recipients. Blood 2010;116:5476–85, doi:http://dx.doi.org/10.1182/blood-2010-04-259291.