MAJOR ARTICLE

Coronavirus 229E–Related Pneumonia in Immunocompromised Patients

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Coronaviruses strains 229E and OC43 have been associated with various respiratory illnesses ranging from the self-resolving common cold to severe pneumonia. Although chronic underlying conditions are major determinants of severe respiratory virus infections, few data about coronavirus-related pneumonia in immunocompromised patients are available. Here we report 2 well-documented cases of pneumonia related to coronavirus 229E, each with a different clinical presentation. Diagnosis was made on the basis of viral culture and electron microscopy findings that exhibited typical crown-like particles and through amplification of the viral genome by reverse transcriptase–polymerase chain reaction. On the basis of this report, coronaviruses should be considered as potential causative microorganisms of pneumonia in immunocompromised patients.

Pulmonary complications occur frequently in immunocompromised patients who are treated for hematological malignancies, and they cover a wide range of etiologies, including infections of bacterial, fungal, parasitic, or viral origin; pulmonary edema; diffuse alveolar hemorrhage; drug- or radiation-induced toxicity; graft-versus-host disease; and bronchiolitis obliterans [1]. In addition, an entity named "idiopathic pneumonia syndrome," defined as diffuse lung injury for which the etiology is not identified, is frequently recognized after hematopoietic stem cell transplantation (HSCT). A large number of these cases of pneumonia may be related to unrecognized viral infections, and, in the absence of routine screening or reliable diagnostic procedures, this number is probably an underestimate.

Coronaviruses are positive-sense, single-stranded RNA viruses whose particles are irregularly shaped. The outer envelope carries distinctive club-shaped peplomers, giving a crown-like appearance. Two coronavirus strains, OC43 and 229E, are known to be involved in human diseases, mainly in the common cold syndrome, but also in pneumonia [2–5]. Coronaviruses recently became a subject of particular interest because a novel strain was identified as the primary agent associated with the worldwide outbreak of severe acute respiratory syndrome (SARS) [6]. We report 2 well-documented cases of coronavirus-related pneumonia in immunocompromised patients who were treated for hematological malignancies. Our diagnostic strategy combined viral culture of HUH7 cells and RT-PCR controlled by hybridization with strain-specific probes.

PATIENTS AND METHODS

Case study 1. A 33-year-old white man was admitted to the hospital with fever, weight loss, and continuous nonproductive cough. Three years previously, this patient was treated for stage II large B cell non-Hodgkin lymphoma, according to the French LNH93 protocol [7]. Because of disease progression, he underwent high-dose chemotherapy intensification supported by autologous HSCT. Long-term, complete remission was achieved. Relapse occurred 2 years after transplantation and was treated with high-dose sequential salvage

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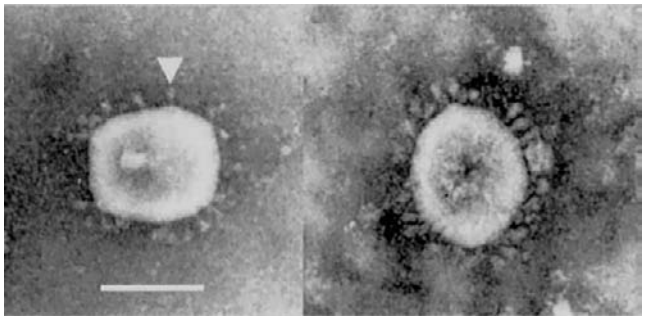

Figure 1. Electron micrograph of culture medium from the bronchoalveolar lavage–fluid specimen obtained from patient 1. Note the presence of coronavirus particles with thin, club-shaped spikes *(arrowhead).* Negatively stained with 2% phosphotungstic acid buffered solution, pH 7.0. Line is 0.1 μm in length.

therapy combining etoposide, ifosfamide, mitoxantrone, and cytarabine.

Ten days after the second cycle of chemotherapy, disseminated cutaneous vesicles appeared, suggesting varicella zoster virus (VZV)-associated eruptions. No respiratory symptoms or abnormal chest radiograph findings were present. The patient was successfully treated with intravenous acyclovir (10 mg/kg every 8 h for 21 days). After completion of acyclovir treatment, the patient developed a febrile nonproductive cough without dyspnea. A chest radiograph showed bilateral interstitial syndrome, and this was confirmed by a CT scan, which showed disseminated micronodular opacities. Arterial blood gas values while breathing in room air were normal. Other laboratory findings were as follows: WBC count, 2100 cells/mm^3, with 1600 polymorphonuclear cells and 500 lymphocytes; platelet count, 20,000 platelets/mm^3. Liver enzyme levels were normal. Sputum samples were negative for *Legionella* species when subjected to direct immunofluorescence, and bacterial culture of the sputum samples showed no growth. Results of tests of serum samples for *Aspergillus* galactomannan antigen were also negative. Following failure of empirical antibiotic treatment (piperacillin and ciprofloxacin), fiber-optic bronchoalveolar lavage (BAL) was performed in the middle lobe. Cytological analysis of specimens of the BAL fluid showed 290 nucleated cells/mm^3, with 18% polymorphonuclear cells, 40% macrophages, and 42% lymphocytes (identified as T-cell lymphocytes by flow cytometry analysis [37% $CD4^+$ cells and 50% $CD8^+$ cells]). The findings of extensive microbiological direct examinations and cultures remained negative for bacteria; mycobacteria; fungi; and parasites, such as *Pneumocystis carinii.* The results of immunofluorescent assays for multiple respiratory viruses (respiratory syncitial virus [RSV]; parainfluenza viruses I, II, and III; adenovirus; and influenzae viruses A and B) and herpes viruses (herpes simplex virus [HSV], VZV), and immunoperoxydase staining for cytomegalovirus (CMV) were negative in specimens of BAL fluid. The result of a PCR test performed on specimens of BAL fluid with VZV-specific primers was also negative [8].

Viral cultures showed no growth in human amniotic cells or in MRC5 and Vero cell lines. In contrast, a cytopathic effect was observed in the human hepatoma HUH7 cell line cultured with specimens of the BAL fluid, which was unrecognized with influenza and parainfluenza viruses, RSV, adenovirus, measles, enterovirus, or VZV-specific antibodies. Electron microscopy findings identified corona-like particles in the culture supernatant medium (figure 1). An RT-PCR test performed with infected and noninfected HUH7 cells and using strain-specific primers located in the M gene of human coronaviruses confirmed the diagnosis and typed the virus as 229E strain, but not as OC43 strain (figure 2). RT-PCR products were also characterized as sequences of 229E virus by hybridization with a specific probe in a DNA enzyme immunoassay [9]. The presence of coronavirus RNA in BAL fluid specimens was confirmed by amplification of 229E sequences, whereas the results of RT-PCR performed with oligonucleotide primers derived from the M gene sequence of the OC43 strain remained negative.

The results of cytopathic effect seroneutralization testing showed a low initial antibody response but a significant difference between the antibody response of preinfection serum samples (titer, <10) and that of postinfection serum samples (titer, 40). All serological studies for respiratory viruses (adenovirus, myxovirus, paramyxovirus, and RSV), CMV, VZV, Epstein-Barr virus, HSV, and *Mycoplasma* species revealed no significant increase in serum sample antibody titer, and the results of serological testing for *Chlamydiae* bacteria and *Aspergillus* remained negative. The outcome was spontaneously favorable, and the fever disappeared in 5 days without modification of the antibiotic regimen, which was consistent with an improvement of the chest radiograph findings.

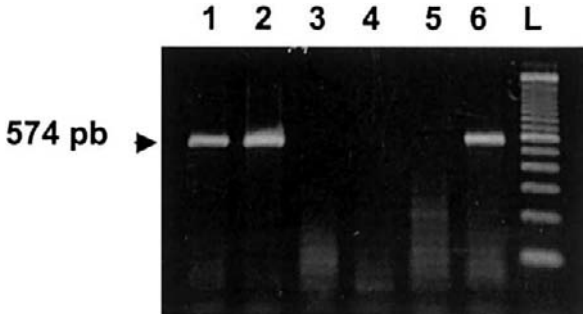

Figure 2. Ethidium bromide stain of a 2% agarose gel, showing RT-PCR amplification products of coronavirus M gene with 229E strain–specific primers. *Lane 1,* Supernatant of the HUH7 cell culture for patient 1. *Lane 2,* Supernatant of the HUH7 cell culture of another patient with common cold. *Lane 3,* Supernatant of noninfected HUH7 cells. *Lane 4,* Negative control extraction. *Lane 5,* Negative control PCR mix. *Lane 6,* Positive control strain 229E grown in MRC5 cells. *Lane 7,* (L) size markers (100 pb).

Case study 2. A 16-year-old female patient was admitted to the intensive care unit (ICU) for acute respiratory failure 5 days after allogeneic bone marrow transplantation. She had been treated with mechlorethamine, vincristine, procarbazine, and prednisone (MOPP) chemotherapy combination 3 years previously for Hodgkin lymphoma with cervical and mediastinal lymphadenopathies. A first relapse with pulmonary and gastric involvement was treated with high-dose chemotherapy intensification (with melphalan, misulban, and cytarabine) supported by autologous HSCT. Because of a second relapse with pulmonary and gastric involvement, the patient underwent allogeneic bone marrow transplantation with a conditioning regimen combining high-dose cyclophosphamide and fludarabine. After initiation of the conditioning regimen, she presented with bilateral knee arthritis. Samples of the joint fluid tested positive for *Aspergillus fumigatus*, and the patient was treated with amphotericin B (1 mg/kg per day). Four days after transplantation, rapidly progressive respiratory failure developed, leading to hospitalization in the ICU.

At admission, physical examination revealed acute respiratory distress with tachypnea (30 breaths/min), bilateral crackles, and oxygen saturation of 98% while breathing with a high-concentration oxygen mask, and shock (heart rate, 140 beats/min; blood pressure, 105/74 mm Hg) with disseminated mottling. Arterial blood gases obtained while breathing oxygen (with a high-concentration oxygen mask at a rate of 12 L/min) were as follows: pH, 7.48; partial pressure of carbon dioxide, 3.15 kPa; partial pressure of oxygen, 19.4 kPa; bicarbonates, 16.9 mmol/L. Laboratory findings revealed pancytopenia (WBC count, 200 cells/mm^3; hemoglobin, 8.5 g/dL; platelet count, 18,000 platelets/mm^3), acute renal failure (creatinine level, 177 μmol/L), and inflammatory syndrome (C-reactive protein, 207 mg/L; fibrinogen, 5.77 g/L). A chest radiograph revealed disseminated alveolar and interstitial opacities.

The patient's condition deteriorated rapidly, requiring endotracheal intubation and mechanical ventilation. Results of a test performed on a tracheobronchial secretion sample were positive for *A. fumigatus*, leading to treatment with a combination therapy of voriconazole and caspofungin. After an initial improvement in clinical characteristics and radiological findings and bone marrow recovery, the patient's respiratory condition worsened, and bilateral alveolar opacities appeared on a chest radiograph. The results of blood tests for *Aspergillus* galactomannan and CMV pp65 antigens remained negative. Specimens obtained by BAL performed in the lingular region were hemorrhagic, and the findings of a quantitative cytological examination were noncontributive, because of numerous lysed cells. The findings of extensive research to detect the presence of microorganisms (bacteria, mycobacteria, fungi, parasites, CMV, and RSV) were negative. Despite aggressive supportive care with mechanical ventilation, fluid resuscitation, and high-dose norepinephrine infusion, refractory hypoxia rapidly led to fatal multiorgan failure. No autopsy was performed. Cultures of BAL fluid specimens remained negative for bacteria, mycobacteria, and fungi. The same procedures for viral culture and RT-PCR were applied as in case study 1, described above. The results of inoculation tests performed with HUH7 cells were also positive, revealing corona-like particles that were subsequently identified as coronavirus 229E by RT-PCR performed on both culture supernatant and BAL fluid specimens.

DISCUSSION

In both cases, 2 major facts led to the diagnosis of coronavirus 229E–associated pneumonia: first, the exclusion of alternative infectious etiologies, and, second, the identification of coronavirus 229E in BAL fluid samples by a combination of culture, electron microscopy, and RT-PCR. In the case of the first patient, the main differential diagnosis was VZV-associated pneumonia, which is the most frequent complication of varicella in adults and is usually concomitant to cutaneous vesicles. However, respiratory symptoms only appeared after completion of antiviral treatment and improvement of skin eruptions, and both viral culture and PCR for VZV performed on BAL fluid specimens were negative. Coronavirus seroconversion retrospectively confirmed the diagnosis.

The second patient presented with severe acute respiratory failure that led to fatal multiorgan failure, despite maximal life support. Ventilator-associated pneumonia is a frequent complication in patients receiving mechanical ventilation, and it is associated with a high mortality rate, especially among bone marrow recipients. Bacterial etiology is largely predominant in ventilator-associated pneumonia, but CMV has been identified as a possible cause in adult patients, and outbreaks of coronavirus- and adenovirus-related pneumonia in pediatric ICUs have been described [10–12]. In the case we describe, though negative results of bacteriological cultures cannot exclude a bacterial origin, a secondary identification of coronavirus in BAL fluid specimens provided a high probability that a diagnosis of viral origin was accurate.

Various methods to detect coronaviruses exist, but they lack sensitivity or specificity when used for routine screening of respiratory samples. These methods include identification of corona-like particles, immunofluorescent assays with monoclonal antibodies, and RNA hybridization in fluid specimens from nasal washing or BAL [13–15]. Detection of seroconversion, based on ELISA findings, may be useful, but it does not allow rapid virus-identification and only provides a retrospective diagnosis [16]. We identified coronavirus in both patients because the BAL specimens were cultured in the human hepatoma HUH7 cell line, which expresses a specific receptor for coronaviruses [17]. Recent advances in virological detection

methods, such as molecular amplification techniques, offer promising perspectives for detection of coronaviruses [18]. Indeed, improved sensitivity and specificity have been obtained with molecular detection methods combining RT-PCR with strain-specific primers derived from the M protein gene, followed by molecular hybridization with specific probes recognizing either coronavirus 229E or OC43 [9]. Inversely, the high sensitivity of amplification methods may lead to false-positive results caused by contamination of BAL fluid specimens with pharyngeal viruses. Therefore, the combination of viral culture and RT-PCR of BAL fluid samples appears to be an efficient and reliable diagnostic strategy.

Two coronavirus strains, 229E and OC43, have previously been related to human diseases with various clinical syndromes, ranging from self-resolving common cold to pneumonia [2–5]. Recently, the worldwide outbreak of SARS was linked to a novel coronavirus that had not been previously identified in human beings or animals [6]. The clinical features of SARS combine flu-like symptoms, dry cough, and shortness of breath, associated with pulmonary infiltrates visible by chest radiography. In contrast to the common benign symptoms of coronavirus infections in healthy individuals, a large proportion of patients with SARS have severe respiratory failure requiring ventilatory support in the ICU. The impact of a respiratory virus on individuals is largely determined by their underlying conditions, and particularly by whether they are experiencing immunosuppression [19, 20]. The prevalence of coronavirus pulmonary infections among immunocompromised patients is unknown, and it is probably largely underestimated in the absence of the routine performance of sensitive cell culture, RT-PCR, or electron microscopy on BAL fluid specimens. However, RT-PCR results were negative for coronaviruses in the BAL samples of 46 HSCT recipients with acute pulmonary infiltrates [21]. Thus, only 1 case of coronavirus-associated pneumonia was previously described in an immunocompromised patient following autologous bone marrow transplantation, with the diagnosis based on the presence of viral particles in BAL fluid specimens [22]. The identification of coronavirus in high-risk immunocompromised patients may lead to early adoption of a specific therapeutic strategy, but, in the absence of proof of the efficacy of antiviral drugs, the treatment remains only symptomatic. In these circumstances, different types of IFNs that display antiviral properties against coronaviruses may be evaluated [23, 24].

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