J Infect Chemother 25 (2019) 543–546

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: http://www.elsevier.com/locate/jic

Case Report

Antiviral treatment and virological monitoring of oseltamivir-resistant influenza virus A(H1N1)pdm09 in a patient with chronic B lymphocytic leukemia☆

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ARTICLE INFO

Article history:
Received 23 August 2018
Received in revised form
16 October 2018
Accepted 12 November 2018
Available online 20 April 2019

Keywords:
Influenza virus
A(H1N1)pdm09
Oseltamivir resistance
Zanamivir
Triple-combination antiviral drug therapy (TCAD)
Chronic lymphocytic leukemia

ABSTRACT

We report the virological monitoring and the antiviral therapy adopted for the treatment of a patient affected by chronic B lymphocytic leukemia, who experienced a severe pneumonia with long-term shedding of influenza virus A(H1N1)pdm09, characterized by an early development of oseltamivir resistance.

1. Introduction

Influenza virus infection remains one of the main public health concerns worldwide, due to its morbidity and mortality. After the 2009 pandemics, awareness towards influenza virus diagnosis and monitoring increased. The Microbiology and Virology Unit of the Padova University Hospital acts as the Italian Veneto Region reference laboratory for the diagnosis of influenza and other respiratory diseases and is in charge for monitoring cases of severe influenza virus infection.

2. Case report

February 3, 2015, a sixty-nine-year-old female patient, affected by chronic B lymphocytic leukemia (B-CLL), was admitted at the Infectious Diseases Division of the Padova University Hospital, with a respiratory tract infection characterized by cold, cough, and arthromyalgia. The computed axial tomography (CAT) indicated the presence of dense nodules, peribronchial thickening with bilateral focal distribution (Fig. 1A). Blood analysis displayed neutropenia and metabolic alkalosis.

A diagnosis of influenza virus A(H1N1)pdm09 infection was achieved by using a real time reverse-transcription polymerase chain reaction (rRT-PCR) assay on the patient nasal swab (Fig. 2). In particular, the viral RNA was extracted from the specimen by using the MagNA STARlet System (Roche) automated platform. Standardized controlled rRT-PCR reaction was performed, as previously described [1], by adopting the SuperScript™ III One-Step RT-PCR System kit along with Platinum™ Taq DNA Polymerase (Invitrogen).

Treatment of B-CLL with Ibrutinib was suspended upon diagnosis of influenza A(H1N1)pdm09 infection to increase the antiviral immune response.

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https://doi.org/10.1016/j.jiac.2018.11.008
1341-321X/

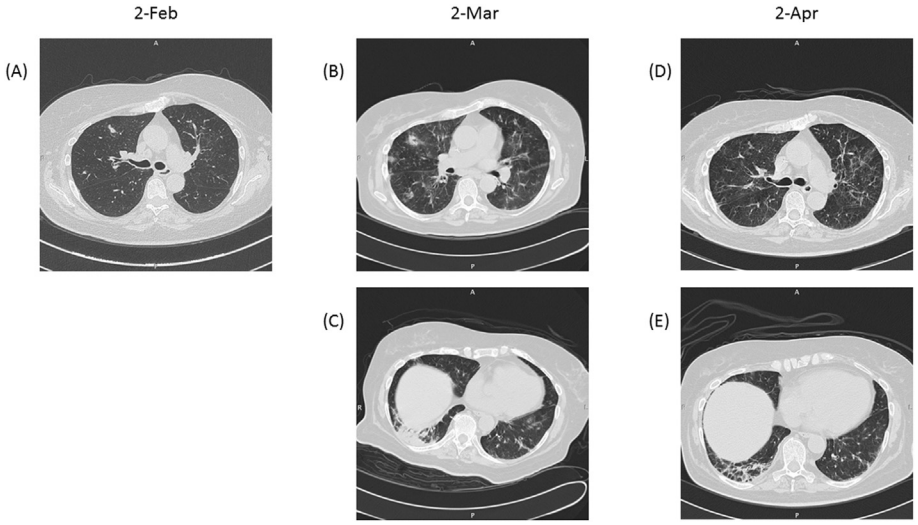

Fig. 1. Results of the computed axial tomography performed at different time points, as specified, to monitor pneumonia evolution. (A) Dense nodules (the larger is >7 mm in diameter) in the right upper lobe, in the ventral portion; peribronchial thickening with bilateral focal distribution. (B) Different thickening and consolidation areas in the upper and middle lobes and (C) consolidation area at the right pulmonary base. (D) Bilateral reduction in the extension of the thickening areas in the upper lobes and (E) resolution of the right lung base thickening.

February 4, oseltamivir treatment (Tamiflu, Roche, 2 × 75 mg/day) was started (Fig. 2) while February 5 Meropenem (Astra Zeneca), 3 × 1 g/day and Azitromycin (Pfizer), 1 × 500 mg/day were added to Bactrim (Roche), 1cp/day, whose administration had initiated already one week before hospital admission (Fig. 3). However, no improvement of clinical signs was observed. By contrast, the patient developed a severe bilateral interstitial pneumonia requiring oxygen therapy with a face mask, followed by a continuous positive airway pressure (C-PAP) treatment. Taking into account the clinical signs, February 9, a broncho-alveolar lavage was

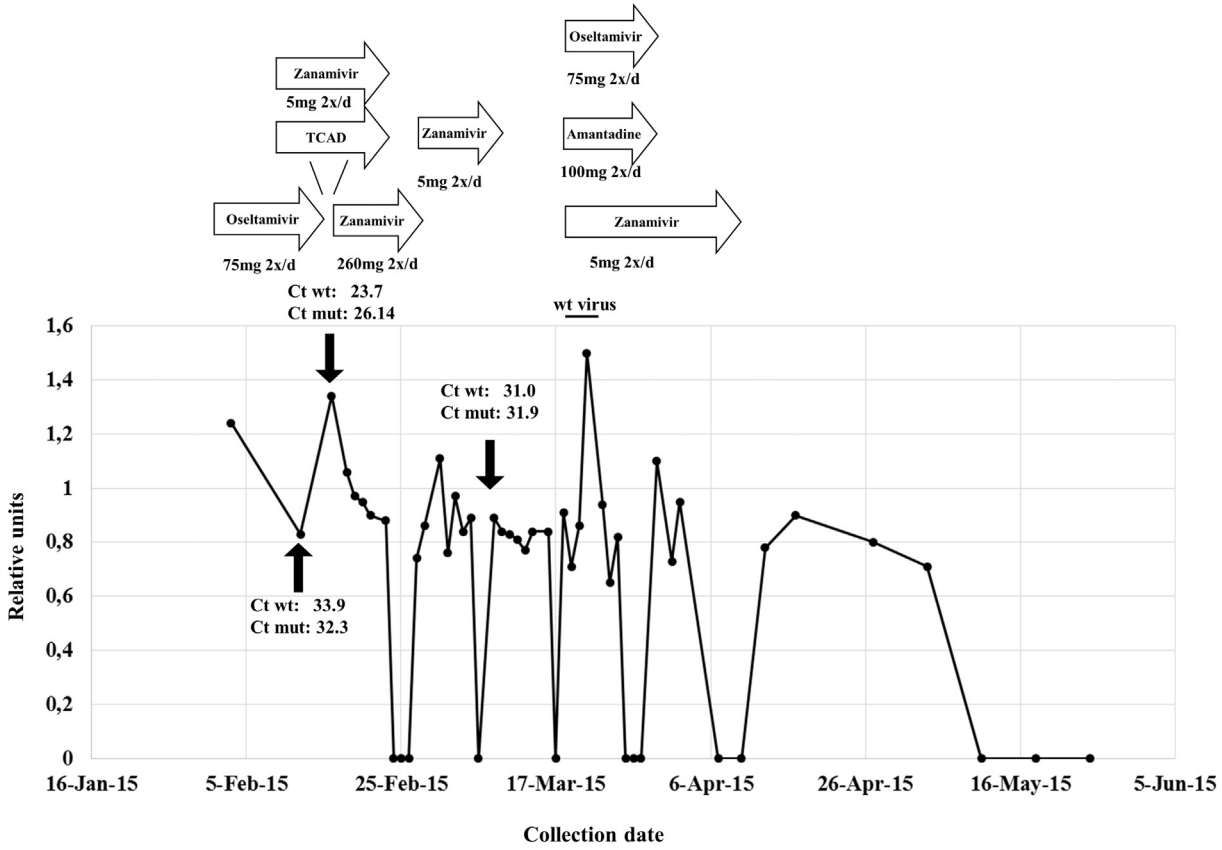

Fig. 2. Virological monitoring and antiviral treatments. Results of rRT-PCRs are expressed as relative unite of Influenza A RNA normalized with respect to the housekeeping gene RNaseP. Continue bar stands for influenza A wild type virus. Ct wt: stands for rRT-PCR threshold cycle for influenza A wild type virus. Ct mut: stands for rRT-PCR threshold cycle for influenza A H275Y mutant virus. TCAD: triple-combination antiviral drug [amantadine (2 × 100 mg/day), ribavirin (4 × 200 mg/day), and oseltamivir (2 × 75 mg/day)].

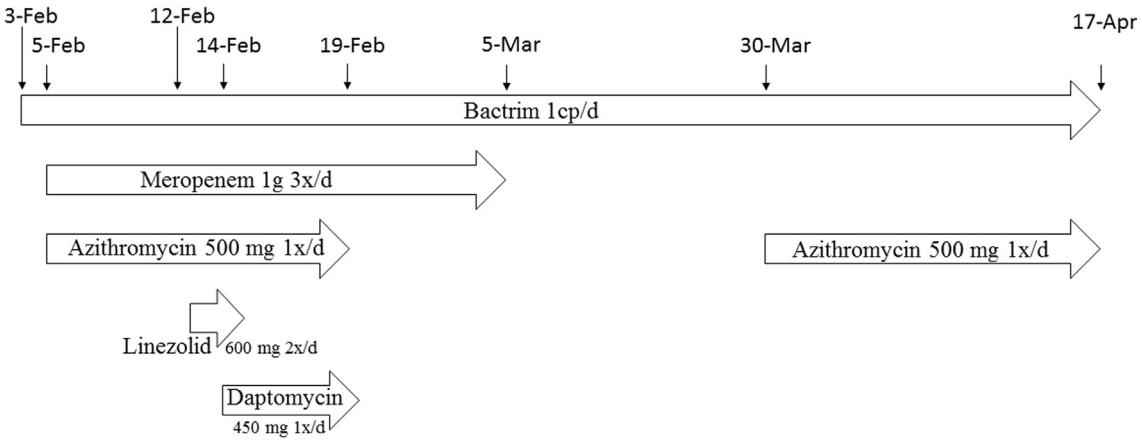

Fig. 3. Timeline and regimen of the prophylactic antibiotic treatment.

collected for further analysis. rRT-PCR resulted positive for A(H1N1) pdm09 influenza virus while the microbiological tests did not show the presence of fungi or clinically relevant bacteria. Indeed, a wide broad antibiotic coverage was started to prevent bacterial super-infection using Linezolid (Pfizer) and Daptomycin (MSD), as reported in Fig. 3.

Furthermore, the patient was constantly monitored for invasive fungal infection with the Platelia™ *Aspergillus* antigen test (BioRad) and for potential bacterial nosocomial infections.

February 12, due to the lack of clinical improvement, a second nasal swab was collected and the virus was analyzed for the presence of mutations conferring drug-resistance. The H275Y mutation within the neuraminidase (NA) viral genomic segment was detected by using a specific rRT-PCR protocol [2]. In particular, the assay indicated the presence of a mixed population of wild type and mutated virus (Fig. 2).

Zanamivir aqueous solution was requested to GlaxoSmithKline (GSK) for intravenous treatment. Meanwhile, a triple-combination antiviral drug (TCAD) therapy - based on the use of amantadine (2 × 100 mg/day), ribavirin (4 × 200 mg/day), and oseltamivir (Tamiflu, Roche, 2 × 75 mg/day) – along with inhaled zanamivir (Relenza Rotadisk, GSK, 2 × 5 mg/day) was administered for three days, to temporary control viral replication. After getting the approval of the local Ethic Committee (Comitato Etico Azienda Ospedaliera di Padova – N. 0009647 – 20/02/2015), an intravenous zanamivir treatment (2 × 600 mg/day) was started, and carried out for ten days (Fig. 2). Twenty-four hours post-first intravenous zanamivir treatment, fever completely resolved with a progressive improvement of pneumonia symptoms and a gradual reduction of the oxygen supplementation need. The rRT-PCR indicated a progressive decrease of A(H1N1)pdm09 viral load in nasal swabs with a complete negativization after five days of treatment with intravenous zanamivir (Fig. 2). However, February 27, following three consecutive negative results, the patient nasal swab resulted positive again for the A(H1N1)pdm09 virus. To achieve viral clearance, zanamivir (Relenza Rotadisk, GSK, 2 × 5 mg/day) was administered by inhalation for additional nine days. Nevertheless, in the follow-up, while the rRT-PCR provided only one positive result for the H275Y mutant virus (March 9), the results for the wild-type virus were constantly positive, with the exception of one sample processed March 17. In addition, March 2, the CAT indicated the presence of different thickening and consolidation areas at the upper and middle lobes (Fig. 1B), as well as a consolidation area at the right pulmonary base (Fig. 1C).

Taking into account the persistence of the wild-type virus, a new cycle of antivirals was undertaken for ten days, based on oseltamivir (Tamiflu, Roche, 2 × 150 mg/day per os), inhaled zanamivir (Relenza Rotadisk, GSK, 2 × 5 mg/day), and amantadine (2 × 100 mg/day), followed by inhalated zanamivir alone for additional ten days. A reduction of the viral yield over the time was obtained, achieving a negative result at day 89 post patient admission. The 2nd of April, CAT showed an improvement of lung alterations (Fig. 1D,E).

Virological monitoring was performed until day 101, indicating a complete viral clearance associated with a full patient recovery from pneumonia.

3. Discussion

Antiviral therapy for the treatment of influenza disease is mainly based on the neuraminidase inhibitors oseltamivir and zanamivir. However, the emergence of resistant viral mutants has been described for both drugs [3]. Furthermore, it has been previously suggested that TCAD might be clinically useful in the treatment of influenza viruses that are resistant to one or more antivirals [4,5]. Thus, there is still space for new drugs acting on different molecular targets (such as the polymerase) of influenza virus.

The H275Y mutation is the most commonly observed change associated with resistance to oseltamivir, and it frequently emerges and persists upon oseltamivir administration in influenza A(H1N1) pdm09 positive immunocompromised patients [6]. Our previous observations and literature data [6,7] suggest that antiviral resistance arises early during treatment. Thus, to optimize the outcome of antiviral treatments it might be useful to routinely test the emergence of the most common resistance-associated mutations in immunocompromised patients, by using rapid and specific assay [8]. Under this respect, the H275Y rRT-PCR we adopted here [2] might represent a valid option.

Furthermore, although up to date the only licensed route of administration for zanamivir is inhalation, the rapid recovery from fever and improvement of clinical signs we observed in our patient support the efficacy of its intravenous administration, at least for the treatment of hospitalized patients with severe influenza. Although there are very little data about the efficacy and mechanism of action of the TCAD regimen, we cannot rule out a contribution of the short TCAD treatment adopted before the intravenous zanamivir administration in the improvement of clinical signs. Zanamivir treatment led to a faster clearance of the H275Y variant (which responds to Zanamivir treatment) with respect to the wild-type virus. This finding is in agreement with literature data [6,9,10] and suggests that mutated virus has a reduced replicative capacity compared to the wild-type virus.

In conclusion, our experience suggests that treatments based on the combination of two (oseltamivir and zanamivir) or more antiviral drugs might help in speeding up clinical signs improvement and viral clearance in immunocompromised patients affected by severe influenza. Further studies are needed to support this conclusion.

Conflicts of interest

We declare no potential conflicts of interest.

Acknowledgments

This work was supported by the Regione Veneto [Centro Regionale Specializzato per la genofenotipizzazione ed epidemiologia molecolare degli agenti da infezione per la diagnostica microbiologica e virale. DGR n. 2154 del 30.12.2015 to GP].

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