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Virological and serological analysis of a recent Middle East respiratory syndrome coronavirus infection case on a triple combination antiviral regimen

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ABSTRACT

Serological, molecular and phylogenetic analyses of a recently imported case of Middle East respiratory syndrome coronavirus (MERS-CoV) in Greece are reported. Although MERS-CoV remained detectable in the respiratory tract secretions of the patient until the fourth week of illness, viraemia was last detected 2 days after initiation of triple combination therapy with pegylated interferon, ribavirin and lopinavir/ritonavir, administered from Day 13 of illness. Phylogenetic analysis of the virus showed close similarity with other human MERS-CoVs from the recent Jeddah outbreak in Saudi Arabia. Immunoglobulin G (IgG) titres peaked 3 weeks after the onset of illness, whilst IgM levels remained constantly elevated during the follow-up period (second to fifth week of illness). Serological testing confirmed by virus neutralisation assay detected an additional case that was a close contact of the patient.

1. Introduction

An upsurge of Middle East respiratory syndrome coronavirus (MERS-CoV) infection has been recently described in countries of the Arabian Peninsula resulting in exported cases from these countries to the European Union [1]. Cases of MERS-CoV infection are associated with a high case fatality rate since there is no available treatment. There is a scarcity of data on specific therapeutic interventions for the disease. Published reports propose the use of known antivirals based on extrapolation of data from: (i) the severe acute respiratory syndrome (SARS) epidemic that was also associated with the circulation of a novel coronavirus; (ii) in vitro data; (iii) animal experimental infections and therapy data; and (iv) limited clinical data for actual MERS-CoV infections [2–4]. However, no clear-cut recommended therapeutic regimen exists and the evidence for grading such interventions is generally low, with the exception of the use of convalescent serum that based on biological effects is given the highest grade [5]. Moreover, little is known about the viral kinetics of MERS-CoV-associated infection, especially when a specific antiviral or other therapeutic intervention is attempted.

A case of MERS-CoV has recently been described in Greece in a traveller who had extensive contact with the healthcare environment in Jeddah (Saudi Arabia) [6]. Here we describe molecular, serological and phylogenetic analyses of this case as well as evidence for a second case that was a close contact of the first patient. Furthermore, we provide evidence of the kinetics and the pattern of viral excretion in biological specimens obtained from the first Greek case while the patient was on a triple antiviral regimen.

2. Methods

2.1. Case investigation

A preliminary report of the first imported, laboratory-confirmed MERS-CoV case in Greece has been described elsewhere [6]. A full description of the course of illness and treatment regimen in

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relation to kinetics of virus shedding and immune response was prepared by review of the patient records. In the course of the outbreak investigation, 40 of 75 patient's contacts, including the patient's wife, provided an oropharyngeal sample for PCR testing 1 week after contact with the positive case; 5 additional contacts were included in the serology examination group. All were submitted to personal clinical monitoring for fever and upper respiratory infection symptoms and were advised to call the Hellenic Centre for Disease Control and Prevention (CDC) command centre immediately in such an instance. In addition, all were offered the chance to provide serum samples on a voluntary basis for specific anti-MERS antibody testing at baseline (same time as the oropharyngeal PCR testing) and 3 weeks after exposure.

2.2. Laboratory evaluation

During the patient's stay in the intensive care unit (ICU), samples from the oropharynx, trachea, urine and faeces were tested for diagnostic evaluation and to monitor viral shedding. A real-time reverse transcription PCR (RT-PCR) method based on amplification of the upstream Envelope gene (*upE*), the nucleocapsid (N) gene and the open reading frame (ORF) 1a of the virus was used for detection of MERS-CoV according to previously described methodology [7,8].

Immunoglobulin G (IgG) and IgM antibody titres in serum samples were determined using an anti-MERS-CoV Indirect Immunofluorescence Assay (Euroimmun AG, Lübeck, Germany). Confirmation of the serological findings was performed with a virus neutralisation assay as described previously [9].

Samples from the patient's upper respiratory tract underwent conventional or molecular testing for the presence of other respiratory pathogens: thus, cultures applied for bacterial testing, whilst real-time RT-PCR was performed for several respiratory viruses including influenza A and B viruses, respiratory syncytial virus (RSV), parainfluenza, adenovirus, enterovirus, bocavirus and human metapneumovirus (hMPV) (M.W.S. r-gene; bioMérieux, Marcy-l'Étoile, France). Specific urine antigen testing of urine samples was utilised for *Legionella pneumophila* and *Streptococcus pneumoniae* (BinaxNOW®; Alere, Orlando, FL). A stool culture was performed due to a history of possible typhoid fever, diagnosed by treating physicians in Saudi Arabia [6].

2.3. Phylogenetic analysis

Nucleotide sequences of 3-kb concatenated sequences of representative MERS-CoVs were analysed and a phylogenetic tree was constructed by the PhyML method as described previously [10].

3. Results

3.1. Case description

A 69-year-old patient of Greek origin who was a permanent resident of Jeddah presented to a tertiary care centre a few hours after arriving in Athens (Greece) on 17 April 2014. His chief complaints included fever since 8 April 2014 and diarrhoea since 10 April 2014. The most likely source of exposure was the hospital environment in Jeddah. The patient had no known co-morbidities. At the time of initial evaluation, a fever of 38.3 °C was noted together with low oxygen saturation (92%), although the patient exhibited minimal respiratory symptoms. A chest radiograph depicted bilateral lung infiltrates consistent with viral pneumonia. The patient was immediately placed under isolation because of suspicion of MERS-CoV infection, and an antimicrobial regimen targeting community-acquired pneumonia was initiated.

On 18 April 2014, MERS-CoV infection was confirmed by means of viral RNA detection in a pharyngeal swab at the Department of Microbiology, University of Athens Medical School (Athens, Greece).

After laboratory confirmation of MERS-CoV, the patient was transferred to a specialised respiratory disease unit in the 'Sotiria' Chest Diseases Hospital of Athens where he was treated in a negative pressure regular room in isolation until 20 April 2014 when, due to deterioration of his respiratory function and development of acute respiratory disease syndrome (ARDS), he was intubated, ventilated and transferred to a negative pressure room in the ICU of the same hospital. An empirical antiviral regimen was initiated on Day 13 of illness consisting of oral (p.o.) lopinavir/ritonavir (400/100 mg twice daily), pegylated interferon (180 μg subcutaneously once per week for 12 days) and ribavirin (2000 mg p.o. loading dose, followed by 1200 mg p.o. every 8 h for 8 days) based on available evidence [3–5,11,12] (Fig. 1).

The patient remained intubated exhibiting hypoxia and occasionally hypercapnia while breathing inspired oxygen in the range of 0.45–0.60. He remained febrile with a plateau temperature of >39 °C and a maximum value of 40.5 °C on Day 18 of illness. Fever started subsiding below 38 °C on Day 22. Acute kidney injury was diagnosed on Day 16 of illness and rapidly progressed to non-oliguric renal failure that reverted to RIFLE injury level (i.e. two-fold increase in the serum creatinine, or glomerular filtration rate decrease by 50%, or urine output <0.5 mL/kg/h for 12 h) on Day 21. The patient's diarrhoea resolved gradually starting on Day 13 and he developed constipation thereafter with normalisation of his bowel movements and gastrointestinal function on Day 19. Owing to development of jaundice and hyperbilirubinaemia attributed to ribavirin [13], the drug was discontinued on Day 20. During the course of his hospitalisation, the patient was diagnosed with adenocarcinoma of the colon and eventually died from septic shock 2 months and 19 days after the initial diagnosis.

3.2. Testing for other pathogens

Cultures and antigen detection were negative for *L. pneumophila* and *S. pneumoniae*. Virological testing was negative for the presence of any other respiratory virus. No relevant enteric pathogens were identified as a cause of the patient's diarrhoea.

3.3. MERS-CoV testing and shedding

RNA was detected in several consecutive patient samples from different sites that included faecal material and serum (Fig. 1). Shedding of MERS-CoV in the respiratory secretions of the patient was noted until the fourth week of illness, whereas viraemia was last detected 15 days after onset of illness and 2 days after initiation of the triple combination antiviral regimen. Consecutive urine testing did not reveal the presence of MERS-CoV RNA (Fig. 1).

3.4. Serological testing for MERS-CoV and new possible case

Serological testing showed a peak IgG titre during the third week of illness, whilst during the fourth and fifth weeks IgG titres were substantially declining. IgM titres were persistently elevated during the whole survey period (Day 13 until Day 34 of illness) (Fig. 1). Viral neutralisation assays performed at Erasmus Medical Center (Rotterdam, The Netherlands) confirmed the immunofluorescence testing results.

Initial and follow-up serological testing were performed on serum samples from 45 patient's contacts. Seroconversion was revealed in one of them who developed an IgG titre of 1/500 and an IgM titre of 1/100 at 21 days after making contact with the patient. This was a 63-year-old man with a past medical history of coronary artery heart disease and diabetes. The presence of specific