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## Case Report

# Human coronavirus NL-63 infection in a Brazilian patient suspected of H1N1 2009 influenza infection: Description of a fatal case

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## ARTICLE INFO

## Article history:

Received 9 July 2011

Received in revised form 9 September 2011

Accepted 12 September 2011

## Keywords:

Human coronavirus-NL63

Respiratory tract infection

Fatal outcome

## ABSTRACT

Human coronaviruses (HCoVs) cause upper respiratory tract and occasionally lower respiratory tract diseases. The recently described human coronavirus NL63 has not been well investigated among Brazilian patients. We reported the clinical course of an HCoV-NL63 infection in a hospitalised patient suspected of H1N1 2009 infection during the second pandemic wave of influenza activity. A 46-year-old female, health care worker with diabetes and presenting with influenza-like illness (ILI) was admitted to the hospital. During 9 days of influenza-like symptoms, the patient had diabetes decompensation, haemorrhagic pneumonia, rhabdomyolysis, respiratory and renal failure, pericarditis, and brain edema and died. HCoV-NL63 may be a causative agent of previously unexplained respiratory illnesses.

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## 1. Why this case is important?

Acute respiratory illnesses (ARIs) are a major health problem in people of all ages, exerting a large economic burden on the health care system. Human coronaviruses (HCoVs) are one family of viruses associated with respiratory tract infections.<sup>1</sup> Five HCoVs are currently known to infect the respiratory tract: HCoV-OC43 and HCoV-229E were first identified in the late 1960s and are well recognised as important pathogens that cause the common cold. In 2003, human coronaviruses received worldwide attention with the emergence of severe acute respiratory syndrome (SARS) caused by a novel coronavirus (SARS-CoV). The increase in research on these viruses soon led to the discovery of two other human coronaviruses: in 2004, HCoV-NL63 was described in the Netherlands, and, in 2005, HCoV-HKU1 was described in China.<sup>2,3</sup> Previous reports have documented HCoV-NL63 infections worldwide and have revealed that infection occurs without gender, age or geographic boundaries and can account for 5% of all acute respiratory infections in the hospital, especially during the winter.<sup>4–11</sup> The most frequently observed clinical manifestations in HCoV-NL63-infected patients are fever, cough, coryza, sore throat, bronchiolitis, bronchitis, pneumonia and croup.<sup>4</sup> We described the clinical pattern of severe respiratory disease with a fatal outcome caused by HCoV-NL-63 in a Brazilian adult patient hospitalised at Hospital São Paulo (a tertiary-university health center). The patient was origi-

nally suspected of having an H1N1 2009 infection during the second pandemic wave of the influenza activity.

## 2. Case report

### 2.1. Case description

In June 2010, a female 46-year-old health care worker, who had previously used tobacco (25 years-pack) but had not done so in the last 6 years and who had been diagnosed with diabetes mellitus type II and been on insulin pump treatment (30 U/day) since 2009, was hospitalised due to suspicion of H1N1 2009 influenza infection. She had been referred to the emergency room 5 days of fever (40 °C), productive cough, coryza, sore throat and inappetence. In addition, the patient had one day of dyspnea, vomiting, abdominal pain and diarrhea. The physical examination on admission was as follows: 35 °C axillary temperature; heart and respiratory rates of 130 bpm and 32 per min, respectively; high blood pressure (170/100 mmHg); blood oxygen saturation by pulse oximetry of 90%; and capillary glycemia of 400 mg/dl. Lung evaluation revealed alteration of breath sounds (shortness of breath at the left base and diffuse rhonchus); and the chest radiograph showed bilateral pulmonary infiltrates, without signs of consolidation or effusion. No other signs or symptoms of additional organ involvement were noted. A lower respiratory tract infection of probable viral aetiology was diagnosed.

Laboratory tests performed in the first 48 h revealed the following: leukocyte count of 25,400 cell/mm<sup>3</sup>, haemoglobin of 17.3 g/dl, haematocrit 52.8%, C-reactive protein level of 385 mg/l (normal value: <10 mg/l) and creatine phosphokinase level of 7995 U/l (normal value: 26–140 U/l). Measurement of arterial blood gas

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**Table 1**  
Laboratory and clinical findings.

	Admission	5th day admission	Discharge
<b>Laboratory findings</b>			
On venous blood			
Hb	17.3 g/dl	11.3 g/dl	10.0 g/dl
Ht	52.8%	31.3%	28.7%
WBC	25,400 cell/mm <sup>3</sup>	34,100 cell/mm <sup>3</sup>	46,000 cell/mm <sup>3</sup>
On arterial blood			
pH	7.05	7.21	6.88
pCO <sub>2</sub>	20.4 mmHg	53.5 mmHg	131 mmHg
pO <sub>2</sub>	70.5 mmHg	159 mmHg	125 mmHg
HCO <sub>3</sub>	5.4 mmol/l	20.8 mmol/l	23.3 mmol/l
BE	−24.8 mmol	−6.6 mmol	−11.6 mmol/l
SatO <sub>2</sub>	41.7%	98.7%	95.8%
<b>Clinical findings</b>			
Axillary temperature	35 °C	38.2 °C	37.7–39.9 °C

Hb, haemoglobin; Ht, haematocrit; WBC, white blood cell count.

tensions showed ketoacidosis (pH: 7.05; pCO<sub>2</sub>: 20.4 mmHg; pO<sub>2</sub>: 70.5 mmHg; HCO<sub>3</sub>: 5.4 mEq/l; BE: −24.8; SatO<sub>2</sub>: 41.7%). Serological tests were negative for HIV, HBV and HCV. Laboratory tests were performed again upon hospital admission (Table 1). A hypothesis of rhabdomyolysis was also considered. The initial treatment was hydration, insulin time-pump (50 UI-intravenous), ceftriaxone (2 g per day), clarithromycin (500 mg twice a day) and oseltamivir (150 mg per day). On Day 1 after admission, due to a worsening of the patient's breathing pattern, lowered consciousness and hypotension, the patient was admitted to the ICU, received a vasopressor drug and was maintained with oxygen therapy followed by tracheal intubation with spontaneous purulent drainage. Cefepime and steroids treatments were then introduced. Positive and expiratory pressure (PEEP) was 12 mmHg, and FiO<sub>2</sub> was 40%. The Glasgow scale registered 15. The patient's condition escalated requiring increased doses of intravenous insulin and PEEP parameters. An acute renal failure occurred, and the anuric patient underwent a haemodialysis procedure. Glycemic peaks were observed, and vasopressor drugs were administered. On Day 3 after admission, due to a worsening of the clinical and ventilator parameters, a bronchoscopy was performed and showed haemorrhagic pneumonia. *Staphylococcus* spp. were cultured from the tracheal aspiration specimen, but blood cultures were negative. Blood gas analysis revealed improvement of the metabolic levels compared to initial values when ketoacidosis was diagnosed (pH 7.41; pCO<sub>2</sub>: 35 mmHg; pO<sub>2</sub>: 136 mmHg; HCO<sub>3</sub>: 22.1 mEq/l; BE: −1.6). An echocardiogram was performed and revealed pericardial effusion without signs of endocarditis. Findings upon physical examination revealed polyarthritides and edema in the upper extremities, but negative results were obtained for nuclear antibodies (ANAs). On Day 8 after admission, the patient had two cardiac respiratory arrests (6 min each) due to hypoxia that were reversed. Severe hypotension without response to noradrenalin and other interventions was irreversible. During hospitalisation, laboratory and clinical findings revealed leukocytosis and fever (Table 1). A CT-scan of the patient's skull was performed and demonstrated brain edema. On Day 9 after admission, the patient was declared brain dead. Virological investigations

A bronchoalveolar lavage (BAL) was collected on the day of admission. RNA and DNA were extracted using QIAamp Viral RNA Mini Kit and QIAamp DNA Mini Kit (Qiagen, Hombrechtikon, Switzerland), respectively. A real-time PCR assay with primers and probes for influenza A, swine flu A, swine H1 and RNaseP (SuperScript III, Invitrogen) were performed following the protocol recommended by the Centers for Disease Control and Prevention (CDC).<sup>12</sup> The viral targets of the real-time PCR assay were: influenza A gene M (matrix) and influenza swine H1N12009 gene

HA (hemagglutinin) and gene NP (nucleoprotein). After a negative result for influenza virus was obtained, RT-PCR assays targeting other respiratory viruses (respiratory syncytial virus A and B, human coronaviruses NL63, HKU-1, 229E and OC43, human metapneumovirus and rhinovirus) were performed using protocols previously described<sup>9,13,24,25,27</sup> and PCR was performed to detect adenovirus following protocols previously described.<sup>26</sup> Only HCoV-NL63 was detected in the samples after RNA amplification. Sequencing was performed to confirm the specificity of the RT-PCR assay using the pancoronavirus primers as previously described<sup>9</sup> with an ABI PRISM BigDye Terminator Cycle Sequencing Reaction kit on an ABI PRISM 3100 DNA sequencer (Applied Biosystems) according to the manufacturer's instructions. The obtained sequences were compared with the prototypical HCoVs sequences available in the GenBank database (NC.005831, AY563107-8 and AY758276-82<sup>2,30</sup>) release 142.0 using BLAST analysis (NCBI BLAST server), which confirmed that the virus detected within the sample was HCoV-NL63.

### 2.3. Other similar and contrasting cases in the literature

Previous reports have demonstrated that the HCoV-NL63 is found in upper and lower respiratory tract infections among children, causing symptoms and signs that do not differ greatly from the symptoms described for the 'old' viruses HCoV-229E and HCoV-OC43, such as fever, cough, rhinorrhea, bronchiolitis and primarily croup.<sup>1,5,8,17,18,21</sup> The majority of these data include clinical descriptions of HCoV-NL63 infection in children. To our knowledge, there are few published case reports regarding adult HCoV-NL63 infection and outcomes. In this sense, the clinical features and the fatal outcome of our diabetic patient with a documented HCoV-NL63 lung infection cannot be compared to others case reports commonly describing coronavirus infection among children patients.

Researchers in Denmark reported a fatal lower respiratory tract disease with human coronavirus NL63 in an adult haematopoietic cell transplant recipient which 5 months after transplantation the patient developed an influenza-like illness and was hospitalized. The patient died 5 weeks after admission owing to progressive respiratory failure and no other additional organ involvement were noted.<sup>29</sup>

## 3. Discussion

HCoV-NL63 is one of the four human coronaviruses that circulates worldwide. The occurrence of HCoV-NL63 among adults and the clinical course of this infection have not been fully elucidated. This report is the first that describes the clinical pattern of HCoV-NL63 infection in a hospitalised Brazilian adult with a fatal outcome.

Recently, a comprehensive three-year study using a multiplex real-time assay revealed 0.64% of HCoV-NL63 in patients from Edinburgh, United Kingdom, being the second human coronavirus most frequently found.<sup>31</sup> Researchers in China reported that 0.1% of HCoV-NL63 infection in adults presented with acute respiratory illness of which 12.5% of infected patients presented with lower respiratory tract infections. The most common clinical symptoms found in coronavirus-infected patients were headache, muscle pain, sore throat, chills and runny nose.<sup>14</sup> Another study reported one HCoV-NL63-infected elderly Canadian patient who died 5 days after the onset of disease, showing that the severity of the respiratory disease can be substantial.<sup>19</sup> The involvement of other organ systems that are non-respiratory is still controversial but can occur, for example the enteric and nervous systems can be involved.<sup>15,28</sup>

Some studies have shown that human coronaviruses infections in patients presenting with underlying conditions, such as diabetes, can cause more severe respiratory tract disease and require hospitalisation.<sup>20,22,23</sup>

According to the medical history and laboratory findings of our report, ketoacidosis, haemorrhagic pneumonia and respiratory failure were documented in a coronavirus-infected patient with an HCoV-NL63 infection. This infection is implicated in a more severe outcome than a mild viral respiratory disease. Rhabdomyolysis, diagnosed by an elevated creatine phosphokinase level and the renal impairment, was also documented. Rhabdomyolysis has been described among patients with others viral infections, such as Influenza A and SARS-associated coronavirus. The cause of acute renal failure could be associated with pre-renal factors, for example hypotension, rhabdomyolysis and the previously documented diabetic condition. An echocardiogram revealed pericarditis, showing that the virus had possibly reached the pericardial space via blood viremia following a respiratory tract infection. Due to no serum available, we could not test for HCoV-NL63 since a positive signal would have strengthened the hypothesis of NL63-involvement in a systemic infection.

Many groups have reported the occurrence of coinfections with HCoV-NL63 and other respiratory viruses.<sup>13,16,17</sup> In our case, no other viruses were detected, including influenza H1N1 2009.

Our results suggest that HCoV-NL63 can be a relevant pathogen causing severe lower respiratory tract infections. This aetiology should be investigated among at-risk adults patients hospitalised with suspected viral pneumonia.

### Conflict of interest

The authors have no conflict of interest to declare.

### Acknowledgments

This project was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo, Brazil (FAPESP) (No. 09/17307-6). This study was approved by the Ethics Committee of Sao Paulo Federal University (CEP 1968/09), and written consent was obtained from the patient's family.

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