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Severe A(H1N1)-associated Pneumonia Sequential to *Clamidophila pneumoniae* Infection in Healthy Subject

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Abstract. *Background: Pandemic influenza virus has been implicated in serious lower airways illness and death in subjects both with and without underlying medical conditions . Predictive factors for severe disease in healthy individuals have not been identified. Case Report: Severe A(H1N1)-associated pneumonia occurring in a healthy subject without underlying medical conditions sequential to a Clamidophila pneumoniae infection, is reported. Conclusion: A potential synergistic mechanism by which other pathogens could interfere with the clinical course of A(H1N1) infection, is suggested.*

Pandemic 2009 influenza A(H1N1) virus infection has been shown to affect lower airways causing serious illness and death in patients with underlying medical conditions such as asthma, diabetes, heart, lung and neurological diseases and pregnancy. Similar outcomes have been reported in previously healthy individuals although predictive factors for severe disease in this patient group have not been identified (1).

Whilst the site of initial infection is likely to be the upper airway, in certain hosts with unrecognized predisposing factors or for possible variants of 2009 A(H1N1) virus strains, the primary site of active viral replication may be the lower respiratory tract.

Clinical observations have identified underlying medical conditions known to increase the risk of severe influenza or influenza complications (2, 3) in 74% of patients, although the remaining 26% experiencing severe disease did not have any co-morbidity (4). During pandemic influenza A(H1N1) 12-17% of adult individuals without underlying medical conditions have experienced severe disease requiring hospitalization (1, 4).

The role of co-infections in A(H1N1) severe illness is unclear. Two early reviews of severe cases of 2009 pandemic influenza A(H1N1) showed no evidence of bacterial pneumonia among hospitalized patients (5,6). Postmortem lung specimen examination from 77 patients with fatal A(H1N1) pandemic influenza infection showed evidence of concurrent bacterial infection in 29% of the cases (7). However, in this study, not all the potential pathogens were evaluated (including intracellular respiratory microorganisms), patient information was incomplete and assessments of bacterial co-infections were performed at autopsy and hence inadequate tissue sampling and prolonged antimicrobial treatment before death may have affected the identification of bacteria; therefore no firm conclusion could be drawn about whether the cause of death was the influenza virus, bacterial infection, or both.

Case Report

In November 2009, during the peak of 2009 A(H1N1) pandemic influenza in southern Italy, a 64-year-old man presented to outpatients with sudden onset of symptoms of severe dyspnoea, dry cough, fever (temperature 39.5˚C), chills, diffuse myalgia and malaise. His blood pressure was 135/80 mm Hg, pulse 112 beats per minute, respiratory rate 30 breaths per minute and oxygen saturation was 90% in ambient air. Physical examination revealed coarse crackles throughout the right lung. Chest radiography showed parenchymal consolidation in the right upper lobe and opacities in the right lower lobe consistent with a diagnosis of multilobar pneumonia (Figure 1).

The patient was referred to the regional centre for 2009 pandemic A(H1N1) influenza. Influenza A (H1N1) virus

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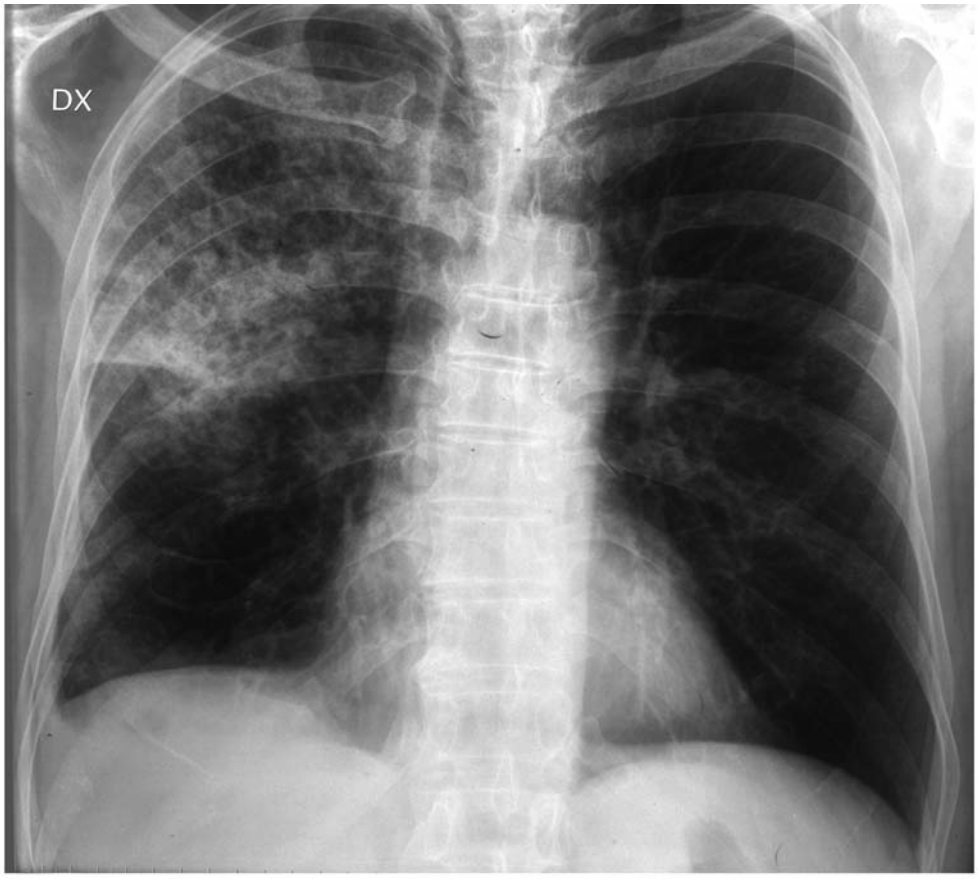

Figure 1. *Parenchymal consolidation in the right upper lobe and opacities in the right lower lobe at baseline chest X-ray.*

infection was laboratory-confirmed by using a real-time reverse-transcriptase–polymerase-chain-reaction assay (FAST SET H1N1v Arrow Diagnostics s.r.l., Genova, Italy).

Oseltamivir, antibiotics (chephalosporin and fluorquinolones for two weeks) and oxygen supplementation was administered; mechanical ventilation was not required. Two weeks later, after completion of therapy, clinical remission of symptoms was achieved and significant, but incomplete resolution of areas of consolidation was observed by chest X-ray (Figure 2). A complete resolution of consolidation detected by chest computed tomography scan (data not shown) was achieved six months later.

The clinical history of the patient did not reveal any underlying medical conditions such as obesity, diabetes hypertension or any respiratory, renal, cardiac or neurological disorders.

Interestingly, four months prior to the influenza AH1N1 diagnosis, the patient had been referred to our outpatients with a two month history of episodic dry cough and tickly throat without evidence of clinical or radiological involvement of lower airways; spirometry was normal and skin prick test was negative for common allergens. Diagnosis of *Clamidophila pneumoniae* infection was performed following serological microimmunofluorescence test (MIF) for respiratory intracellular pathogen infections which was positive for both *Clamidophila pneumoniae* IgM and IgG antibodies.

After a sequential course of levofloxacin followed by azythromycin antibiotics, a complete remission of the clinical symptoms was achieved and chest X-ray confirmed the absence of any thoracic involvement. At this stage absence of IgM antibodies, but persistence of IgG for *Clamidophila pneumoniae* was detected.

Discussion

Whilst underlying medical conditions which make individuals susceptible to develop lower airway disease causing serious illness and death have been extensively investigated, predictive factors for severe disease in previously healthy individuals infected with pandemic 2009 influenza A(H1N1) virus have not been clearly identified. During pandemic influenza A(H1N1) rates of hospitalization

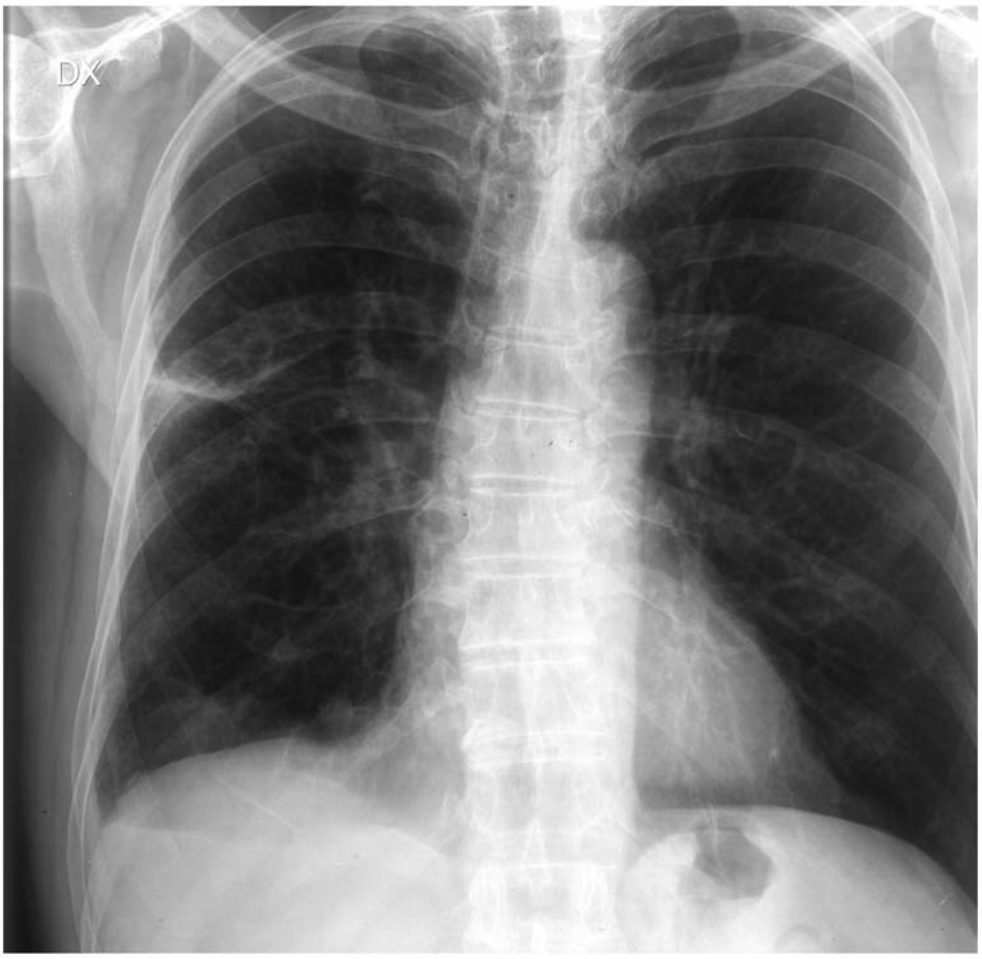

Figure 2. *Partial resolution of the parenchymal consolidation after therapy.*

and death have varied widely according to country; hospitalization rates have been highest for children under the age of 5 years and lowest for persons 65 years of age or older (8); approximately one quarter of adult individuals without underlying medical conditions have experienced severe disease requiring hospitalization (1, 4, 8).

Evidence indicates the importance of bacterial concurrent co-infections in severe A(H1N1) infections (3) although there are no reports on the potential role of sequential infections.

A(H1N1) virus uses a sialyl glycans as receptor for cell attachment which varies in its distribution among tissues of different species and determines host range and tissue tropism as well as the capacity of animal viruses to initiate a human pandemic. Soundararajan *et al*. predicted that the H1N1 2009 virus, A/California/4/2009 (Cal/09) would be able to make optimal contacts with α2-6-linked sialyl glycans, a feature shared with other human H1N1 HAs and, in addition, make contacts with α2-3-linked sialyl glycans (9). Long chain α2-3-linked sialyl (poly-N-acetyllactosamine) sequences are present in human ciliated bronchial epithelial cells; interestingly they are also the receptors for another human intracellular pathogen, *Mycoplasma pneumoniae*.

The present clinical observation suggests a potential synergistic mechanism by which other infections could interfere with the clinical course of A(H1N1) influenza.

Chlamydia pneumoniae is considered the most common non-viral intracellular human respiratory pathogen and has been associated with a wide spectrum of clinical respiratory phenotypes (10,11). There is evidence that intracellular infectious agents such as Clamidophila pneumonia are able to overexpress molecules such as Inter-Cellular Adhesion Molecule 1 (ICAM-1) (12), which act as viral cell receptors for Rhinovirus (13). Although there are no reports on the effects of *Clamidophila pneumoniae* on sialic acids this case suggested a possible synergic action of this microorganism in amplifying the lower respiratory epithelial cells

susceptibility to A(H1N1) infections by mechanisms similar to those involved in *Rhinovirus* receptor amplification.

This case highlights the need to further investigate whether intracellular respiratory infections could represent a possible risk factor for developing influenza-associated severe pneumonia in healthy individuals without underlying medical conditions. Serological assessment of intracellular pathogen in healthy subjects, who develop severe influenza-associated pneumonia without microbiological evidence of bacterial co-infections should be considered.

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