

# Pathology and Pathogenesis of 2009 Pandemic H1N1 Influenza

Wun-Ju Shieh, MD, MPH, PhD  
Infectious Diseases Pathology Branch, Division of Viral and Rickettsial Diseases  
Centers for Disease Control and Prevention

## **BACKGROUND**

Novel influenza A (H1N1) is a new influenza virus of swine origin that first caused illness in Mexico and the United States in March and April, 2009. The novel influenza A (H1N1) virus spreads in the same way as regular seasonal influenza viruses do, mainly through the coughs and sneezes of people who are sick with the virus, but it may also be spread by touching infected objects and subsequently touching one's nose or mouth. Novel H1N1 infection has been reported to cause a wide range of flu-like symptoms, including fever, cough, sore throat, body aches, headache, chills and fatigue. In addition, many people also have reported nausea, vomiting and/or diarrhea. The first novel H1N1 patient in the United States was confirmed by laboratory testing at CDC on April 15, 2009. The second patient was confirmed on April 17, 2009. It was quickly determined that the virus was spreading from person-to-person. On April 22, CDC activated its Emergency Operations Center to better coordinate the public health response. On April 26, 2009, the United States Government declared a public health emergency and has been actively implementing the nation's pandemic response plan.

On June 11, 2009, the World Health Organization (WHO) indicated that a global pandemic of novel influenza A (H1N1) was underway by raising the worldwide pandemic alert level to Phase 6. This action was a reflection of the magnitude of spread of the new H1N1 virus, and not the severity of illness caused by the virus. At that time, more than 70 countries had reported cases of novel influenza A (H1N1) infection. By June 19, 2009, all 50 states in the United States, the District of Columbia, Puerto Rico, and the U.S. Virgin Islands had reported novel H1N1 infection.

Since the WHO declaration of a pandemic, the new H1N1 virus has continued to spread, with the number of countries reporting cases of novel H1N1 nearly doubling. In the Southern Hemisphere's, regular influenza season started concurrently with the new H1N1 influenza in June. In the United States, significant novel H1N1 illness has continued to occur in the summer,

with localized and in some cases intense outbreaks. The United States continues to report the largest number of novel H1N1 cases of any country worldwide, however, most people who contracted novel H1N1 have recovered completely without requiring medical treatment.

Given ongoing novel H1N1 activity to date, the CDC anticipates that there will be more cases, more hospitalizations and more deaths associated with this pandemic in the United States over the winter and early spring. The novel H1N1 virus, in conjunction with regular seasonal influenza viruses, poses a potential threat to the general public due to the significant illness it may cause, especially with associated hospitalizations and deaths during the influenza season. The mortality rate of novel H1N1 is estimated to be around 0.01%. This presentation describes the clinicopathologic, epidemiological, and pathogenetic studies of autopsy tissue specimens obtained from U.S. fatal cases with 2009 pandemic influenza A (H1N1) virus infection.

## **PATIENT CHARACTERISTICS AND LABORATORY TESTS**

One hundred confirmed case-patients with fatal 2009 H1N1 virus infection were evaluated at Infectious Disease Pathology Branch, CDC from May 12 to October 1, 2009. Fifty-three (53%) of these case-patients were confirmed to have 2009 H1N1 by testing postmortem specimens. These 53 cases had no antemortem diagnosis and the confirmatory diagnosis was only obtained from testing postmortem samples. This finding underscores the important role of medical examiners, coroners, and pathologists in infectious disease surveillance by performing postmortem examination and testing autopsy samples.

The median age of fatal case-patients was 36 years, range 2 months to 84 years, 80% were aged 20 - 60 years, and 51 (51%) were male. The majority (85%) of case-patients with known previous medical history had at least one underlying comorbidity. Obesity (46%), cardiovascular disease (25%) and asthma (22%) were the three most frequent conditions reported. Fever (82%), cough (67%) and shortness of breath (58%) were the most common signs and symptoms reported, with a median duration from illness onset to death of 8 days, range 1 to 44 days. Fifty-eight (67%) of 87 case-patients with available clinical history were hospitalized prior to death. Forty-two (74%) of 57 case-patients with available hospital records required mechanical ventilation. Radiographic diagnosis of pneumonia was documented in 59% (38/64) of case-patients.

In the cases with known medical history, obesity (BMI  $\geq$  30) is the most significant associated factor and almost 19% of these patients were extremely obese (BMI  $\geq$  40). There is a clear association between obesity and metabolic disorders; however, very little is known about the effect of obesity on immune function, especially during an infection. It was reported that diet-induced obese mice are more susceptible to morbidity and mortality during influenza infection than lean mice. Obesity may interfere with cellular responses during influenza infection, leading to selective impairment in dendritic cell function and alterations in the T-cell population that may be detrimental to the host. Whether similar altered immune responses also occurred in humans with influenza virus infection are unknown. Nevertheless, obesity is the most common precipitating factor for obstructive sleep apnea as well as a risk factor for the development of asthma. A number of studies also indicate its association with a higher risk of developing deep vein thrombi, pulmonary emboli, pulmonary hypertension, and pneumonia. Further studies are needed to elucidate the pathophysiologic affect of obesity and other medical conditions on patients with 2009 pandemic influenza A (H1N1) virus infections.

The autopsy samples were evaluated with histopathologic examination, immunohistochemical assays (IHC), PCR assays, viral culture, and electron microscopic examination. Of the 100 case-patients with confirmed 2009 H1N1, testing of respiratory tissue by rRT-PCR assays at CDC were positive for influenza A virus in 90, including 87 for H1N1 and 3 non-subtypeable virus. Based on available records for 80 case-patients with known duration of illness, rRT-PCR results were positive for 2009 H1N1 in respiratory tissue specimens of 42 (53%) case-patients with illness duration <10 days and in 28 case-patients (35%) with illness >10 days when death occurred. Negative rRT-PCR results for 2009 H1N1 were not observed in any case-patients with known illness duration <10 days, but results were negative in 7 case-patients (9%) with duration >10 days. In these same 80 case-patients with known duration of illness, positive IHC results for influenza A viral antigen were observed in respiratory tissues of 31 case-patients (39%) with illness duration <10 days and in 5 case-patients (6%) with illness >10 days. In contrast, negative IHC results for influenza A were observed in respiratory tissues of 14 case-patients (18%) with time from onset to death <10 days and in 31 case-patients (39%) who died after an illness >10 days. 2009 H1N1 virus was isolated from fresh lung tissue specimens in five of 30 case-patients tested.

## **HISTOPATHOLOGIC FINDINGS**

The major histopathologic features were observed in airways and lungs. Eighty-five case-patients had airway tissues available for evaluation. The most frequent histopathologic findings in airways were inflammation and edema (66%). The inflammation was usually mild and consisted predominantly of mononuclear cells. Necrosis of epithelium (26%) and hemorrhage (18%) were less frequently observed. Lung tissues in all case-patients showed a spectrum of histopathologic changes of diffuse alveolar damage (DAD). The nature and extent of DAD generally corresponded to the duration of clinical illness of the patients. Forty-one case-patients had paratracheal or hilar lymph nodes available for evaluation and hemophagocytosis was noted in 25 (61%) of these case-patients. Pulmonary thromboemboli were noted in gross autopsy findings or microscopically in 17 case-patients. No histopathologic evidence of myocarditis or encephalitis was observed in any of the case-patients with heart (n=30) or CNS samples (n=19) available for evaluation. Histopathologic findings in other organs were nonspecific and most likely associated with the patients' underlying medical conditions. These findings included prominent eosinophils in patients with history of asthma; enlarged nuclei of cardiac myocytes in patients with history of hypertension; and fatty metamorphosis in the liver in obese patients.

Histopathologic evaluations accentuated DAD as the most significant and consistent finding. This lung involvement is more similar to the histopathologic features in fatal avian influenza (H5N1) and is seen less frequently in fatal seasonal influenza cases. However, a proportion of pandemic influenza A (H1N1) cases in this report also showed inflammation or other histopathologic changes in trachea, bronchi, or bronchioles, a pattern more commonly observed in severe or fatal cases of seasonal influenza.

Other respiratory viral infections can also cause DAD, especially at the end stage of critical illness. Some of these viruses may show distinct cytopathic effects or inclusions in the lung that can be differentiated from influenza virus infection. Examples of these include adenovirus, human herpesviruses, measles virus, respiratory syncytial virus, Nipah virus, and parainfluenza virus. Since these distinct cytopathic effects are not seen in influenza virus infection, a combination of clinical judgment, epidemiological surveillance data, and various laboratory tests are necessary for a reliable diagnosis of influenza.

## **VIRAL LOCALIZATION AND CELLULAR TARGETS**

By using IHC assay with anti-influenza A NP antibody, the viral antigens were observed in respiratory tissues from 44 case-patients (44%); the amount of influenza virus antigen varied, with abundant immunostaining in 9 and rare in 24 case-patients. Viral nucleoprotein antigens were localized in the nuclei and cytoplasm of infected cells, including epithelial cells in airways, submucosal glands, and pneumocytes, either detached or lining alveoli. Antigens were also seen in association with hyaline membranes and in endothelial cells in rare cases. Double staining revealed that the major cellular targets of viral infection were pneumocytes, predominantly type II, and occasionally macrophages. No immunostaining of influenza A viral antigen was detected in any of the non-respiratory tissue samples available. Influenza rRT-PCR testing on a limited number of these non-respiratory samples was also negative. Electron microscopic examination of lung tissue identified rare infected cells with extracellular virus particles in the alveolar space. Virions were round to oblong-shaped and averaged 88 nm in diameter; some particles were surrounded by spikes approximately 12 nm in length.

Immunolocalization showed viral antigens were predominantly in the lung parenchyma. The damage to the lung is associated with localization of viral antigens. The amount of viral antigens varied from case to case, and appeared to be more abundant in cases with shorter duration of illness. This temporal correlation probably denotes the clearance of viral antigens by host immune responses at later stage of illness. IHC with double immunostaining for influenza virus and cell markers, such as surfactant, cytokeratin, and CD68, demonstrated the localization of pandemic influenza A (H1N1) virus antigens is mainly in pneumocytes, especially type II. The involvement of pneumocytes is similar to avian influenza (H5N1) virus infections and other viral infections like SARS, representing the hallmark of severe damage of the alveolar architecture. Type II pneumocytes are known to secrete pulmonary surfactant, which reduces surface tension and preserves the integrity of the alveolar space. These cells also play an important role in tissue restitution after lung damage. Type II pneumocytes can be directly infected by bacteria or viruses and modulate the corresponding pathogenesis of these organisms. Comparing to avian influenza (H5N1) virus infection, the involvement of pneumocytes with pandemic influenza A (H1N1) virus was much more extensive. This observation correlates with the studies of receptor tropism among different influenza A viruses and probably represents more accessible receptors in human lung for pandemic influenza A (H1N1) virus. Viral antigens can also be seen in tracheo-bronchiolar epithelial cells, glandular epithelial cells, and occasional

macrophages. The viral immunolocalizations in airway and lung parenchyma are in agreement with studies of receptor binding demonstrating the ability for 2009 H1N1 virus to target both upper and lower respiratory tract tissue. In cases of seasonal influenza, viral antigens are predominantly present in airway epithelial cells and rarely involve alveolar pneumocytes or macrophages. The differential presence of receptors may account for the dissimilarity of histopathologic changes and viral antigen distribution among seasonal influenza A viruses, avian influenza A (H5N1) virus, and 2009 pandemic influenza A (H1N1) infections.

## **BACTERIAL AND VIRAL COINFECTIONS**

Overall, 26% of case-patients had confirmatory test results of bacterial coinfection. Twenty-nine case-patients showed histopathologic evidence of bronchopneumonia with prominent alveolar polymorphonuclear cells, indicating a possibility of bacterial coinfection. Of these, 22 (76%) case-patients had a specific bacterial pathogen identified. Bacterial agents were identified in an additional 4 case-patients that did not show histopathologic evidence of bronchopneumonia in the tissue sections examined. Gram-positive cocci were the most frequent bacteria identified by using special stains. IHC and PCR assays were positive as follows: 9 case-patients positive for *S. pneumoniae*, 3 case-patients for *S. pyogenes*, 1 case-patient for both *S. pyogenes* and *S. pneumoniae*, 1 case-patient for both *S. pyogenes* and *S. mitis*, 1 case-patient for *S. mitis*, 1 case-patient for *S. agalactiae*, 4 case-patients for MRSA, 1 case-patient for both MRSA and *S. pyogenes*, 1 case-patient for both MRSA and *H. influenzae*, and 4 case-patients for MSSA. None of the case-patients were found to have evidence of a coinfection with RSV, parainfluenza viruses 1-3, or adenovirus.

Bacterial co-infections in severe cases of influenza have been well documented in previous influenza pandemics and in studies of seasonal influenza. For instance, during the 1957-1958 H2N2 pandemic, secondary bacterial pneumonia was considered the most frequent complication of influenza with an occurrence in <75% of fatal cases. Interactions among influenza virus and co-infecting bacterial pathogens often affect the nature and severity of clinical manifestations and disease outcome. As previously reported, it is often difficult to correlate culture results with clinicopathologic features of pneumonia due to multiple confounding situations, such as inconsistency of obtaining samples for culture, contamination of postmortem samples with growth of mixed bacteria, and inherent problems of culture techniques.

IHC assays performed using formalin-fixed, paraffin-embedded tissues offered the advantage of identifying specific respiratory bacteria pathogens in areas with histopathologic evidence of bacterial pneumonia. The IHC positive cases can be further confirmed by panbacterial 16S PCR assay and agent-specific PCR assay. In this series, bacterial pneumonia was present in 29% of cases, with *S. pneumoniae*, *S. aureus*, and *S. pyogenes* as the most frequent agents identified, alone or combined with other organisms. The incidence of *S. aureus*, either MRSA or MSSA, has become alarmingly high, especially among younger patients.

## **PATHOGENESIS**

The consistent presence of DAD in all of the fatal pandemic H1N1 Influenza cases indicates a primary cytopathic effect caused by viral infection. Although immunolocalization does not indicate active viral replication, the finding of viral antigen within pneumocytes and in association with hyaline membranes suggests a direct viral cytopathic effect as a major pathogenic mechanism in this disease. In addition to the primary viral pneumonia, other factors, such as underlying medical conditions and bacteria co-infections as discussed previously may also contribute to the morbidity and mortality of this novel virus infection. Various degrees of hemophagocytosis is present in 15 (65%) of 23 cases with lymph nodes available for examination. Hemophagocytosis is a nonspecific histopathologic finding that can be seen in many infectious diseases. However, it may play a role in pathogenesis of influenza virus infection because of its possible correlation with cytokine-mediated process. Several studies of naturally acquired or experimentally infected influenza demonstrated that respiratory and constitutional symptoms correlate with the presence of multiple cytokines, including IL-4, IL-6 and TNF- $\alpha$ , in plasma or nasopharyngeal fluid, but viremia has not been detected through the disease process. The potential role of these cytokines in the pathogenesis of developing severe or fatal outcome of influenza virus infection needs further investigation.

In summary, the most significant histopathologic feature in fatal cases of 2009 pandemic influenza A (H1N1) is various degrees of diffuse alveolar damage. PCR and IHC assays are instrumental in establishing diagnosis and studying pathogenesis of this emerging viral infection. The immunolocalization of pandemic influenza A/H1N1 viral antigens shows pneumocytes and alveolar lining cells are the most prominent targets involved in the infection. Although there are

limitations for detecting bacteria in postmortem lung samples, a combination of histopathologic evaluation, special stains, IHC assays, and PCR assays provides valuable diagnostic information to identify etiologic bacterial organisms as the source of co-infection. In addition to bacterial co-infection, the severe or fatal outcome of many patients with pandemic influenza A (H1N1) virus infection may be attributed to other underlying medical conditions, such as obesity and asthma. More studies, including experimental animal studies, are needed to further our understanding in the pathogenesis of this emerging influenza virus.

## REFERENCES

1. Dawood FS, Jain S, Finelli L, et al. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. *N Engl J Med* 2009;360:2605-15.
2. Echevarria-Zuno S, Mejia-Arangure JM, Mar-Obeso AJ, et al. Infection and death from influenza A H1N1 virus in Mexico: a retrospective analysis. *Lancet* 2009:[ePublication].
3. Jain S, Kamimoto L, Bramley AM, et al. Hospitalized patients with 2009 H1N1 influenza in the United States, April-June 2009. *N Engl J Med* 2009;361:1935-44.
4. Presanis AM, Lipsitch M, DeAngelis D, et al. The severity of pandemic H1N1 influenza in the United States, April -- July 2009. *PLoS Currents Influenza Version 3* 2009:RRN1042.
5. Hers JF, Mulder J. Broad aspects of the pathology and pathogenesis of human influenza. *Am Rev Respir Dis* 1961;83(2)Pt 2:84-97.
6. Louriá DB, Blumenfeld HL, Ellis JT, Kilbourne ED, Rogers DE. Studies on influenza in the pandemic of 1957-1958. II. Pulmonary complications of influenza. *J Clin Invest* 1959;38:213-65.
7. Oseasohn R, Adelson L, Kaji M. Clinicopathologic study of thirty-three fatal cases of Asian influenza. *N Engl J Med* 1959;260:509-18.
8. Taubenberger JK, Morens DM. The pathology of influenza virus infections. *Annu Rev Pathol* 2008;3:499-522.
9. Guarner J, Paddock CD, Shieh WJ, et al. Histopathologic and immunohistochemical features of fatal influenza virus infection in children during the 2003-2004 season. *Clin Infect Dis* 2006;43:132-40.

10. Kuiken T, Taubenberger JK. Pathology of human influenza revisited. *Vaccine* 2008;26 Suppl 4:D59-66.
11. Gill J, Sheng Z-M, Ely SF, et al. Pulmonary Pathologic Findings of Fatal 2009 Pandemic Influenza A/H1N1 Viral Infections. *Arch Pathol Lab Med* 2010;134:E1-E9.
12. Mauad T, Hajjar LA, Callegari GD, et al. Lung Pathology in Fatal Novel Human Influenza A (H1N1) Infection. *Am J Respir Crit Care Med* 2009:[ePublication].
13. Perez-Padilla R, de la Rosa-Zamboni D, Ponce de Leon S, et al. Pneumonia and respiratory failure from swine-origin influenza A (H1N1) in Mexico. *N Engl J Med* 2009;361:680-9.
14. Soto-Abraham MV, Soriano-Rosas J, Diaz-Quinonez A, et al. Pathological changes associated with the 2009 H1N1 virus. *N Engl J Med* 2009;361:2001-3.
15. Wang R, Sheng ZM, Taubenberger JK. Detection of novel (swine origin) H1N1 influenza A virus by quantitative real-time reverse transcription-PCR. *J Clin Microbiol* 2009;47:2675-7.
16. Guarner J, Shieh WJ, Dawson J, et al. Immunohistochemical and in situ hybridization studies of influenza A virus infection in human lungs. *Am J Clin Pathol* 2000;114:227-33.
17. Guarner J, Packard MM, Nolte KB, et al. Usefulness of immunohistochemical diagnosis of streptococcus pneumoniae in formalin-fixed, paraffin-embedded specimens compared with culture and gram stain techniques. *Am J Clin Pathol* 2007;127:612-8.
18. Shieh WJ, Hsiao CH, Paddock CD, et al. Immunohistochemical, in situ hybridization, and ultrastructural localization of SARS-associated coronavirus in lung of a fatal case of severe acute respiratory syndrome in Taiwan. *Hum Pathol* 2005;36:303-9.
19. Gu J, Xie Z, Gao Z, et al. H5N1 infection of the respiratory tract and beyond: a molecular pathology study. *Lancet* 2007;370:1137-45.
20. Korteweg C, Gu J. Pathology, molecular biology, and pathogenesis of avian influenza A (H5N1) infection in humans. *Am J Pathol* 2008;172:1155-70.
21. Ng WF, To KF. Pathology of human H5N1 infection: new findings. *Lancet* 2007;370:1106-8.
22. To KF, Chan PK, Chan KF, et al. Pathology of fatal human infection associated with avian influenza A H5N1 virus. *J Med Virol* 2001;63:242-6.
23. Uyeki T. Antiviral Treatment for Patients Hospitalized with 2009 Pandemic Influenza A (H1N1). *N Engl J Med* 2009;361:e110.

24. Childs RA, Palma AS, Wharton S, et al. Receptor-binding specificity of pandemic influenza A (H1N1) 2009 virus determined by carbohydrate microarray. *Nat Biotechnol* 2009;27:797-9.
25. Maines TR, Jayaraman A, Belser JA, et al. Transmission and pathogenesis of swine-origin 2009 A(H1N1) influenza viruses in ferrets and mice. *Science* 2009;325:484-7.
26. Munster VJ, de Wit E, van den Brand JM, et al. Pathogenesis and transmission of swine-origin 2009 A(H1N1) influenza virus in ferrets. *Science* 2009;325:481-3.
27. Shinya K, Ebina M, Yamada S, Ono M, Kasai N, Kawaoka Y. Avian flu: influenza virus receptors in the human airway. *Nature* 2006;440:435-6.
28. Qi L, Kash JC, Dugan VG, et al. Role of sialic acid binding specificity of the 1918 influenza virus hemagglutinin protein in virulence and pathogenesis for mice. *J Virol* 2009;83:3754-61.
29. van Riel D, Munster VJ, de Wit E, et al. Human and avian influenza viruses target different cells in the lower respiratory tract of humans and other mammals. *Am J Pathol* 2007;171:1215-23.
30. Pastva AM, Wright JR, Williams KL. Immunomodulatory roles of surfactant proteins A and D: implications in lung disease. *Proc Am Thorac Soc* 2007;4:252-7.
31. Smith AG, Sheridan PA, Tseng RJ, Sheridan JF, Beck MA. Selective impairment in dendritic cell function and altered antigen-specific CD8+ T-cell responses in diet-induced obese mice infected with influenza virus. *Immunology* 2009;126:268-79.
32. Bacterial coinfections in lung tissue specimens from fatal cases of 2009 pandemic influenza A (H1N1) - United States, May-August 2009. *MMWR Morb Mortal Wkly Rep* 2009;58:1071-4.
33. Prevention of pneumococcal disease: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 1997;46:1-24.
34. La Gruta NL, Kedzierska K, Stambas J, Doherty PC. A question of self-preservation: immunopathology in influenza virus infection. *Immunol Cell Biol* 2007;85:85-92.
35. de Jong MD, Simmons CP, Thanh TT, et al. Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. *Nat Med* 2006;12:1203-7.

36. Hui KP, Lee SM, Cheung CY, et al. Induction of proinflammatory cytokines in primary human macrophages by influenza A virus (H5N1) is selectively regulated by IFN regulatory factor 3 and p38 MAPK. *J Immunol* 2009;182:1088-98.
37. Kirkeby S, Martel CJ, Aasted B. Infection with human H1N1 influenza virus affects the expression of sialic acids of metaplastic mucous cells in the ferret airways. *Virus Res* 2009;144:225-32.