CME/SAM

Pathologic Findings in Novel Influenza A (H1N1) Virus ("Swine Flu") Infection

Contrasting Clinical Manifestations and Lung Pathology in Two Fatal Cases

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Key Words: Novel influenza A virus; H1N1 virus; Swine flu; Autopsy; Fatal outcome; Lung pathology; Diffuse alveolar damage; Bronchopneumonia; Bacterial; Oseltamivir (Tamiflu)

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Upon completion of this activity you will be able to:
- discuss basic terminology and nomenclature of influenza virus infection.
- discuss the appropriate autopsy workup for cases of suspected novel influenza A virus infection.
- describe and compare the pulmonary pathology of seasonal influenza virus infection and novel influenza A virus infection.
- list some of the major considerations in the differential diagnosis of diffuse alveolar damage.

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Questions appear on p 510. Exam is located at www.ascp.org/ajcpcme.

Abstract

Although novel influenza A (H1N1) virus infection has assumed pandemic proportions, there are few reports of the pathologic findings. Herein we describe the pathologic findings of novel influenza A (H1N1) infection based on findings in 2 autopsy cases. The first patient, a 36-year-old man, had flu-like symptoms; oseltamivir (Tamiflu) therapy was started 8 days after onset of symptoms, and he died on day 15 of his illness. At autopsy, the main finding was diffuse alveolar damage with extensive fresh intra-alveolar hemorrhage. The second patient, a 46-year-old woman with alcoholism, was found unresponsive in a basement and brought to the hospital intoxicated and confused. Her condition deteriorated rapidly, and she died 4 days after admission. The main autopsy finding was acute bronchopneumonia with gram-positive cocci, intermixed with diffuse alveolar damage. The pathologic findings in these contrasting cases of novel influenza A (H1N1) infection are similar to those previously described for seasonal influenza. The main pathologic abnormality in fatal cases is diffuse alveolar damage, but it may be overshadowed by an acute bacterial bronchopneumonia.

In March 2009, the first cases of human infection by an untypable strain of influenza virus were reported from Mexico and, subsequently, the United States.[1] By early April, the virus was identified as a novel influenza A virus with genetic elements resulting from recombination of swine, human, and avian viruses. The infection was rapidly reported from several other countries worldwide and was deemed a pandemic on June 11, 2009. Although a small number of deaths occurred (mostly in adults between 30 and 50 years old), the majority of reported cases were mild, occurring mainly in people younger than 25 years. At the time of this writing (July 1, 2009), 77,201 cases of novel influenza A (H1N1) virus infection and 332 deaths have been reported to the World Health Organization from multiple nations worldwide, including 27,717 cases and 127 deaths from the United States.[2] The number of cases and deaths in the United States now exceeds that reported from Mexico, although the latter was the first to report rapid spread of the infection. Within the United States, the state with the largest number of deaths so far is New York State (35 deaths), which is the state of residence of the patients in our report.

Despite the large and ever-increasing number of reported cases, there were no reports of the pathologic findings of novel influenza A (H1N1) virus infection at the time of this writing. Since then, 3 case series (one each from Brazil, Mexico, and the United States) have appeared in the literature.[3-5] We recently performed autopsies on 2 people with fatal influenza A (H1N1) virus infection and were struck by the dissimilarity in their clinical manifestations and pulmonary pathology. In view of the public health importance

of this virus, this report attempts to define the pathology of this infection and to compare it with the known pathologic features of seasonal influenza.

Materials and Methods

Two cases of fatal novel influenza A (H1N1) virus infection autopsied at the Onondaga County Medical Examiner's office (Syracuse, NY) in 2009 form the basis of this report. Novel influenza A (H1N1) virus infection was confirmed in both cases by detection of viral RNA by real-time reverse transcriptase polymerase chain reaction (rRT-PCR), performed at the New York State Department of Health Wadsworth Center, which is a referral laboratory approved by the Centers for Disease Control and Prevention (CDC) for confirmatory testing for novel influenza A (H1N1) virus. rRT-PCR is the diagnostic method of choice recommended by the World Health Organization. Next-of-kin of both patients consented to publication of this report.

Results

Case 1

A 36-year-old obese man was admitted to an urgent care center with a 4-day history of cough with yellowish expectoration, fever, wheezing, generalized body aches, malaise, chills, and sweating. His medical history was notable only for hypertension. He smoked 2 or 3 cigars a day and lived with his girlfriend and her 3 children. At admission, he had a temperature of 39.6°C, a heart rate of 124 beats per minute, a respiratory rate of 18 breaths per minute, and a blood pressure of 136/85 mm Hg. His oxygen saturation (measured by pulse oximetry) was 94% to 95% while breathing room air. Crackles were auscultated in both lung bases. A chest radiograph showed a left lower lobe infiltrate. A throat culture was obtained, and he was discharged the same day with amoxicillin-clavulanate.

During the next 3 days, progressive respiratory distress and cough developed. On day 7 (since the onset of his original symptoms), he was admitted again to the same hospital complaining of cough, shortness of breath at rest (increasing on exertion), decreased appetite, headache, backache, and inability to sleep. On examination, his temperature was 39.5°C, with a respiratory rate of 28 breaths per minute and an oxygen saturation (measured by pulse oximetry) of 77% while breathing room air. There was mucoid material in his pharynx. One examining physician auscultated crackles in both lung bases. A chest radiograph showed extensive bilateral confluent airspace disease in the mid to lower lung fields bilaterally, worse on the left, with small bilateral pleural effusions. The radiographic picture was significantly worse than the prior study. A computed tomogram of the chest showed scattered patchy areas of parenchymal density bilaterally with scattered interstitial changes. Therapy was started with azithromycin and ceftriaxone.

The next day, infectious disease consultation was obtained, whereupon it was learned that his girlfriend's 3 children all had flu-like symptoms a week before his symptoms, and that the school they attended had a recent documented case of H1N1 viral infection. The children had not been tested for influenza, and their symptoms resolved spontaneously. On obtaining this history, influenza A (H1N1) viral infection was suspected in our patient, and a nares specimen was submitted for an influenza A and B screen, which was reported as negative. Oral oseltamivir (Tamiflu) therapy was initiated on day 8 (75 mg orally, twice a day). His oxygen saturation deteriorated to 80% with oxygen by face mask, and he was transferred to intensive care for worsening dyspnea and hypoxia. He was intubated the following day. Sputum cultures grew only *Candida* (considered a contaminant) and were negative for bacterial organisms including *Legionella*. Direct fluorescent antibody testing for *Legionella* and *Pneumocystis* and urine testing for *Legionella* antigen were also negative. On day 12, a nasal aspirate sent to a local laboratory was reported negative for influenza A and B by enzyme immunoassay and was referred to the New York State Department of Health Wadsworth Center for further testing.

Despite therapy, the patient's condition continued to deteriorate, and he died on day 15 of his illness. On the day the patient died, the New York State Department of Health Wadsworth Center reported the nasal aspirate specimen as positive for influenza A (H1N1). An autopsy was performed the same day. Two samples of lung tissue, one sample each of heart and brain, and a nasal swab taken at autopsy were sent to the New York State Department of Health Wadsworth Center for influenza testing. Six days after the autopsy, the nasal swab was confirmed as positive for novel influenza A (H1N1) viral RNA by rRT-PCR, and 1 sample of lung tissue was reported as equivocal. The other sample of lung, as well as the brain and heart samples, were negative for novel influenza A (H1N1). Viral cultures were requested, but were not performed at the reference laboratory.

Autopsy Findings

At autopsy, the lungs were congested, consolidated, and heavy (combined lung weight, 2,925 g) **Image 1A**. Histologically, the main finding was diffuse alveolar damage (DAD) in the acute stage, characterized by extensive hyaline membrane formation **Image 1B** and **Image 1C** affecting large portions of the sampled parenchyma. This was accompanied by hyperplasia of type 2 alveolar pneumocytes. Focally, there was airspace and alveolar septal fibroblast proliferation, suggesting areas of transition to the organizing stage of DAD. Other areas showed a mild chronic inflammatory infiltrate

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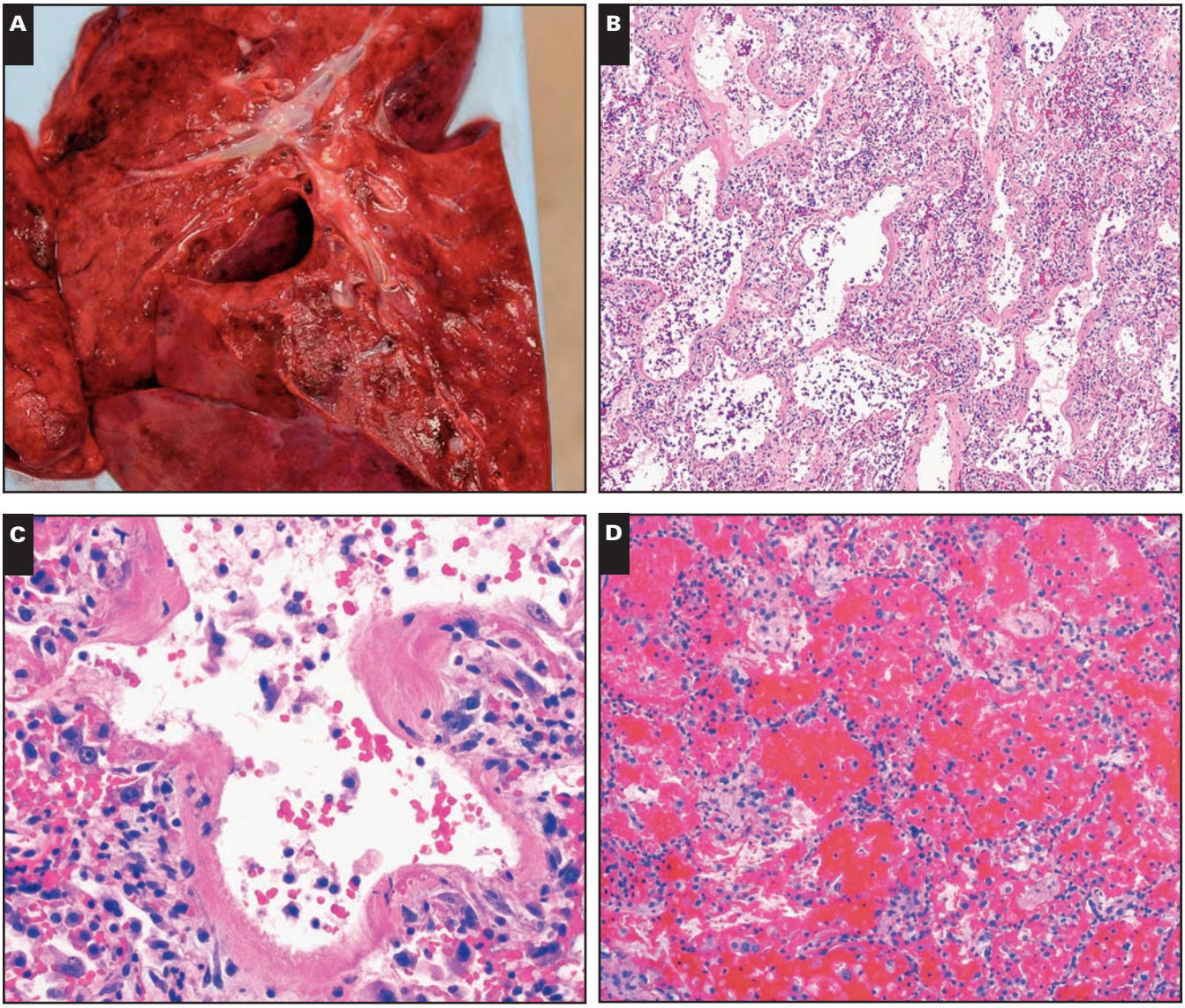

Image 1 (Case 1) Diffuse alveolar damage and intra-alveolar hemorrhage in influenza A (H1N1) virus infection. **A**, Gross image of congested, boggy, and consolidated lung. This macroscopic appearance is abnormal but nonspecific. **B**, Low magnification showing extensive diffuse alveolar damage (DAD) (H&E, ×40). **C**, High magnification of an alveolus lined by a hyaline membrane. Note the absence of viral inclusions (H&E, ×200). **D**, Extensive intra-alveolar hemorrhage (H&E, ×100).

composed of a sprinkling of lymphocytes and plasma cells within interstitium and airspaces, admixed with the DAD. Extensive fresh intra-alveolar hemorrhage was also present **Image 1D**, both associated with and away from the areas of DAD. Small foci of acute inflammation were present in the airspaces and interstitium, but these were not extensive. The bronchi showed mild autolysis and partial sloughing of the respiratory epithelium but no necrosis or ulceration **Image 1E**. One small bronchus contained a tiny focus of squamous metaplasia **Image 1F**. There was minimal and focal chronic inflammation in the bronchial mucosa. Mild chronic inflammation was seen in and around the bronchial submucosal glands, composed predominantly of lymphocytes and an occasional plasma cell. No eosinophils or neutrophils were identified, and there was no basement membrane thickening.

Other autopsy findings included morbid obesity (weight, 332 lb; body mass index, 46.3 kg/m^2), marked edema within subcutaneous tissues, cardiomegaly (heart weight, 525 g), moderate 2-vessel atherosclerosis, and hepatomegaly (liver weight, 2,375 g) with mild steatosis.

Case 2

A 46-year-old homeless woman was brought to an emergency department by a friend, after being found unresponsive in the basement of a building. Eight days previously, she had been treated for a generalized tonic-clonic seizure associated

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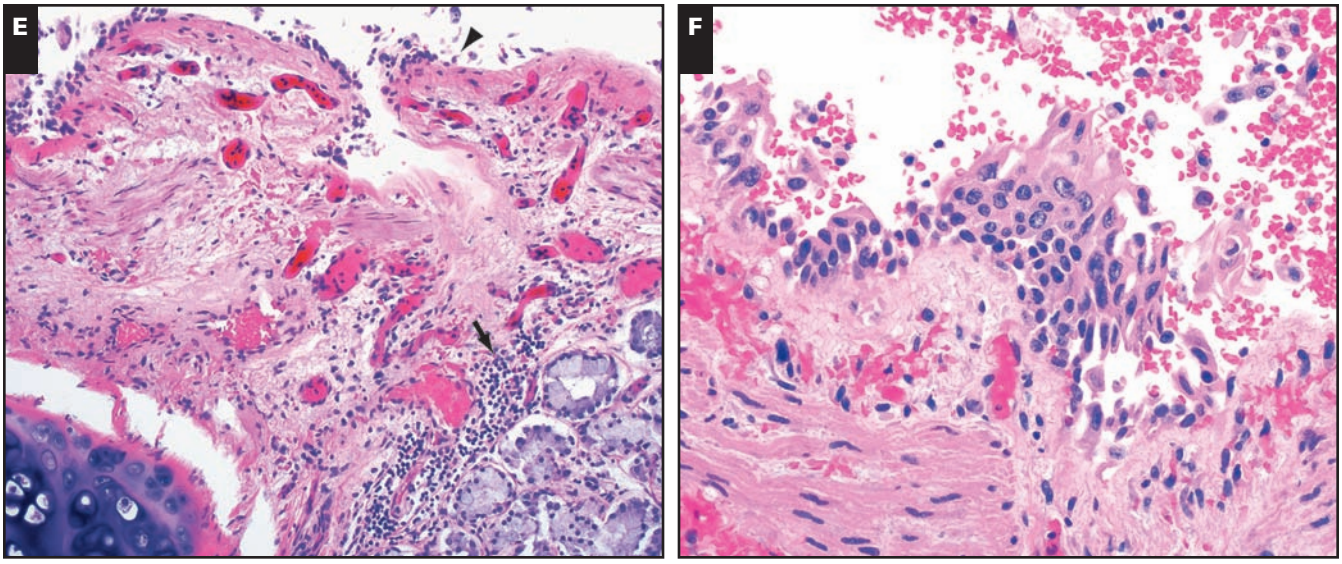

E, Low magnification of the wall of a bronchus. There is mild autolysis in the form of sloughing of the lining epithelium (arrowhead), but no evidence of necrotizing bronchitis. Note the mild chronic inflammation in bronchial submucosal glands (arrow) (H&E, ×100). **F**, A tiny focus of squamous metaplasia within a bronchus (H&E, ×200).

with a blood alcohol level of 50 mg/dL (0.05 g%). Her history was significant for alcohol abuse with multiple admissions for alcohol intoxication and repeated falls, vitamin B_{12} deficiency, hepatitis C, cerebrovascular accident, temporal encephalomalacia, depression, and anxiety.

At admission, she was awake but intoxicated and confused. She was coughing and was also witnessed to have projectile vomiting. She denied dyspnea, fever, and chills. She was covered in feces and found to have bruises on her forehead and buttocks. Vital signs at admission were as follows: temperature, 39°C; heart rate, 144 beats per minute; blood pressure, 112/76 mm Hg; respiratory rate, 18 breaths per minute; oxygen saturation, in the high 80s while breathing room air (90% with 4 L of oxygen by nasal cannula). Coarse breath sounds were auscultated bilaterally. Alcohol intoxication was thought to be the major clinical problem, for which she was given intravenous thiamine and lorazepam. A blood alcohol level was 185 mg/dL (0.185 g%). A total leukocyte count was 4,500/μL (4.5×10^9/L) with 44% band forms. A chest radiograph showed right middle lobe opacification, which was thought to represent aspiration pneumonia. An electrocardiogram showed supraventricular tachycardia. Levofloxacin and intravenous diltiazem were started.

Later that day, she became more delirious and tachycardic and was transferred to intensive care. The next day (hospital day 3), she required intubation. Levofloxacin was discontinued and switched to piperacillin-tazobactam and azithromycin.

On hospital day 4, a sputum Gram stain showed a few gram-positive cocci, and a urine specimen was positive for *Streptococcus pneumoniae* antigen by enzyme immunoassay. Cultures for other bacteria, including *Legionella*, were negative. Clindamycin was started. At the request of the Onondaga County Health Department, a nasopharyngeal swab was sent to the New York State Department of Health Wadsworth Center for influenza A (H1N1) testing. On hospital day 5, trimethoprim-sulfamethoxazole and oseltamivir were started, but the patient's condition continued to deteriorate, and she died.

Results of the nasopharyngeal swab for influenza A (H1N1) were not available at the time of death, and the test was canceled by the referral laboratory after the patient died. An autopsy was performed the next day. Two samples of lung tissue (one from each side) and a nasopharyngeal swab taken at autopsy were sent to the New York State Department of Health Wadsworth Center for influenza testing. Three days after the autopsy, all 3 specimens were confirmed as positive for novel influenza A (H1N1) viral RNA by rRT-PCR. Bacterial cultures of the lung tissue were negative. Further testing at the CDC revealed high viral loads in several of the patient's tissues, and testing for bacterial pathogens in the lung tissue (including immunohistochemical analysis) was positive for *S pneumoniae*. Viral cultures were negative.

Autopsy Findings

At autopsy, there were bilateral pleural effusions (right, 800 mL; left, 700 mL), and both lungs were heavy and consolidated (right lung, 1,260 g; left lung, 1,060 g). Five sections were examined from the lungs, one per lobe of each lung. The most striking abnormality was severe acute bronchopneumonia, characterized by filling of the alveolar spaces infiltrate by neutrophils **Image 2A**. Focally, the neutrophilic infiltrate was

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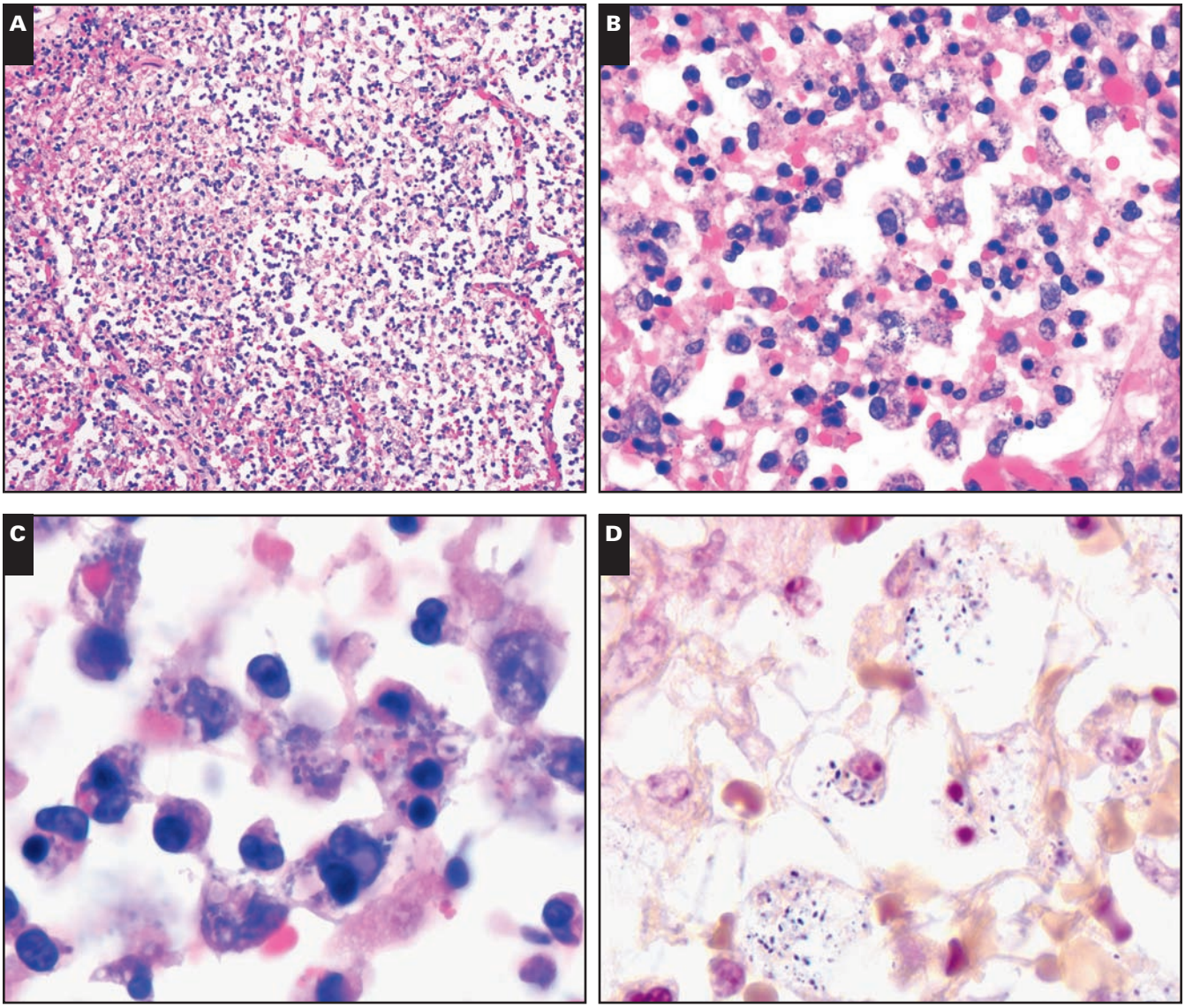

Image 2 (Case 2) Acute bacterial (pneumococcal) bronchopneumonia and influenza A (H1N1)-related diffuse alveolar damage (DAD). **A**, Acute bronchopneumonia, low magnification. Alveolar spaces are filled with a neutrophilic infiltrate and necrotic debris (H&E, ×100). **B**, Acute bronchopneumonia, high magnification. Neutrophils admixed with macrophages (H&E, ×400). **C**, Basophilic granular material within macrophages (H&E, ×1,000). **D**, Gram stain demonstrating that the tiny basophilic granular particles seen in **C** represent gram-positive cocci (Gram/Brown and Brenn, ×1,000).

accompanied by numerous macrophages **Image 2B**, necrotic debris, fibrin, and edema. On H&E-stained sections, some of the macrophages within the areas of bronchopneumonia were seen to contain tiny basophilic granules **Image 2C**. A tissue Gram stain (Brown and Brenn) showed that the small granules within the macrophages were gram-positive cocci **Image 2D**. In some areas, the bronchopneumonia was admixed with areas of DAD **Image 2E**, characterized by hyaline membrane formation. DAD was also present in areas of lung away from the acute inflammation **Image 2F**.

One additional finding was the presence of large numbers of pigmented macrophages within the airspaces, indicative of respiratory (smoker's) bronchiolitis. The overlying visceral pleura showed a few sizable adhesions. The bronchi showed mild autolysis and sloughing of the respiratory epithelium but no necrosis, ulceration, or squamous metaplasia. There was minimal, focal chronic inflammation in the bronchial mucosa and mild chronic inflammation in and around the bronchial submucosal glands composed predominantly of lymphocytes and an occasional plasma cell. No acute inflammation was identified in the bronchi.

Other autopsy findings included hepatic steatosis and a remote cystic infarct in the left temporal lobe of the brain.

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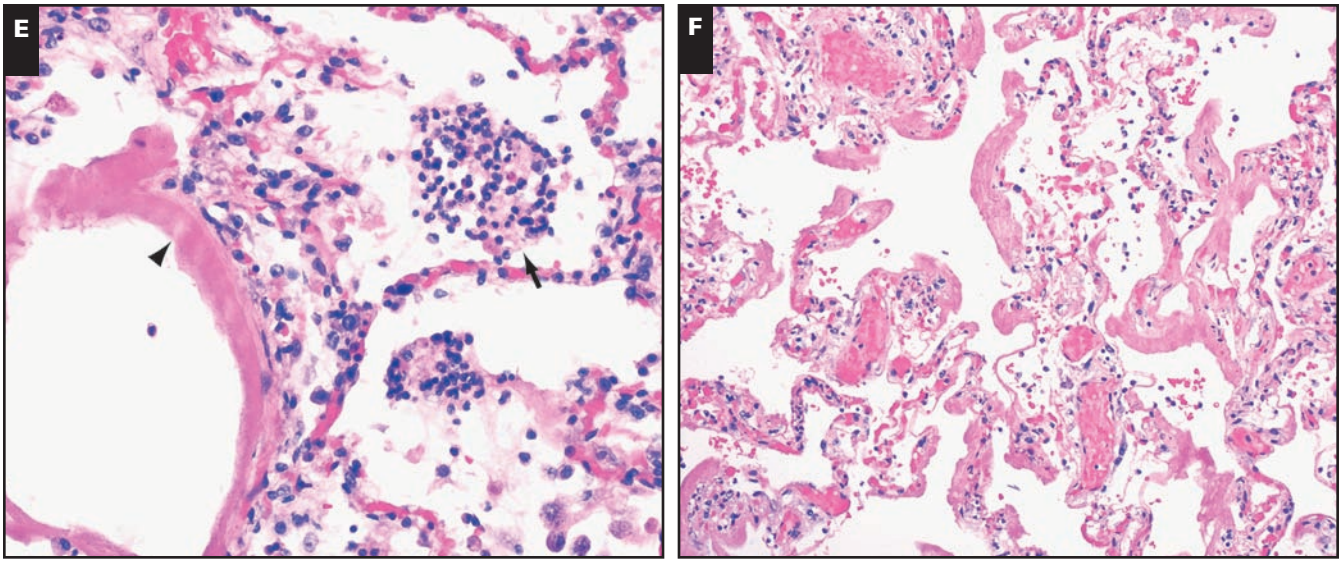

E, DAD (arrowhead) adjacent to acute bronchopneumonia (arrow) (H&E, ×200). **F**, DAD in lung parenchyma away from acute bronchopneumonia (H&E, ×100).

Discussion

Influenza viruses are classified into 3 major genera (A, B, and C),[6] of which influenza A is of greatest clinical significance. Each genus is further classified into serotypes based on the viral proteins hemagglutinin (HA) and neuraminidase (N). Among the various possible subtypes (denoted by combinations of H and N) of seasonal influenza A virus, only 3 (H1N1, H1N2, and H3N2) are currently circulating among humans.[7] Novel influenza A (H1N1) virus (also known as new influenza virus, swine-like influenza virus, swine-origin influenza virus, and colloquially as "swine flu") is a novel form of influenza A virus resulting from a combination of genes derived from 2 types of swine influenza, one of which is, in turn, a "triple reassortant" of human, avian, and swine influenza A strains.[6] This new virus is not only antigenically and genetically distinct from seasonal influenza A (H1N1) virus, but is also usually sensitive to oseltamivir, a neuraminidase inhibitor to which 10.9% of recent seasonal influenza A viruses were resistant.[8]

The pathologic features of (seasonal) influenza pneumonia are well documented.[9-16] Most existing pathologic data on influenza pneumonia are derived from autopsy series, with only a few reports of biopsy findings.[17,18]

The histologic findings of influenza pneumonia can be divided into those caused by the virus and those attributable to superimposed bacterial infection. The two often coexist in the same case. Findings attributed to viral infection include parenchymal and airway abnormalities. The major parenchymal abnormalities are DAD and intra-alveolar hemorrhage. DAD may be seen as early as the second day of illness and as late as day 21.[9,11] It is more prominent in patients requiring mechanical ventilation and supplemental oxygen. Abnormalities in the trachea, bronchi, and bronchioles occur in most cases, the most important being acute necrotizing tracheobronchitis, bronchitis, or bronchiolitis. These changes appear between days 3 and 13 of the illness. Mild chronic inflammation of the airways and degenerative changes of the lining epithelium are also common. Immunohistochemical techniques have demonstrated viral antigens within airway epithelium.[19,20]

Another major finding in the airway mucosa in influenza is squamous metaplasia,[21] which sometimes is so striking as to suggest a neoplastic squamous proliferation.[11,16] Unlike many other viral infections, viral inclusions and viral cytopathic effects are not seen in influenza infection. The major finding attributable to superimposed bacterial infection is acute bronchopneumonia, characterized by a neutrophilic infiltrate within alveolar spaces, accompanied by varying amounts of fibrin and edema. The superimposed bacterium is mostly *Staphylococcus aureus*, *S pneumoniae*, or *Haemophilus influenzae*.

The clinical and pathologic features of the 2 cases presented are strikingly different and should alert clinicians, as well as pathologists, to the heterogeneity of settings in which this infection may be encountered. In our first case, the clinical setting was that of typical flu-like symptoms, and there was a history of contact with children with a flu-like illness. The clinical course was unexpectedly severe, especially for a previously healthy, relatively young patient with no major underlying illnesses. Although oseltamivir therapy was commenced relatively late in the illness (day 8 after onset of symptoms), there may have been some potential benefit even at this stage.

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According to current CDC recommendations, "evidence for benefits from antiviral treatment in studies of seasonal influenza is strongest when treatment is started within 48 hours of illness onset. However, some studies of oseltamivir treatment of hospitalized patients with seasonal influenza have indicated benefit, including reductions in mortality or duration of hospitalization even for patients whose treatment was started more than 48 hours after illness onset."[22]

With regard to pathology, the autopsy findings in case 1 were consistent with a viral pneumonia and fit well with descriptions of "uncomplicated influenza pneumonia" in the literature. The major histologic findings were DAD and intraalveolar hemorrhage, and there was no evidence of superimposed bacterial bronchopneumonia. Necrotizing bronchitis/bronchiolitis and squamous metaplasia, findings frequently seen in seasonal influenza A, were absent or inconspicuous. A specific diagnosis of influenza A (H1N1) viral pneumonia was made only retrospectively, after results of postmortem studies were available.

In contrast, our second case had a distinctly nonrespiratory presentation. Even after radiographic abnormalities were found, they were understandably attributed to aspiration pneumonia rather than a viral process. A similar atypical presentation was described by Oseasohn et al[15] in their series of 33 fatal cases of Asian influenza. One of their patients (case 20) was a 52-year-old woman hospitalized for a head injury sustained when she was intoxicated. The authors made the point that in rare cases, influenza virus is recovered from the lungs of patients lacking premonitory symptoms of a flu-like illness. Pathologically, our second case had typical features of a superimposed bacterial pneumonia. The associated/underlying virus-associated DAD was overshadowed by the bacterial process to such an extent that the DAD was initially thought to be a result of the bacterial pneumonia. If influenza A (H1N1) virus had not been detected by rRT-PCR from the patient's lungs, the presence of the virus-related DAD could have been easily overlooked and attributed to bacterial infection.

Problems in making a rapid clinical diagnosis of novel influenza A (H1N1) infection are readily apparent from these 2 cases. As case 1 illustrates, the diagnosis may not be suspected unless a specific aspect of the history raises this possibility. The resultant delay in diagnosis and therapy may result in an unfavorable outcome. Even with a high index of suspicion, clinical diagnosis is difficult because confirmatory testing takes at least a few days and up to a week. Our second case highlights the difficulty in suspecting novel influenza A (H1N1) infection in patients with atypical clinical manifestations. In such cases, in which there are virtually no clinical clues to suggest a viral process, it may impossible to make a correct diagnosis antemortem. It is safe to state, however, that in the throes of a pandemic, any patient with an unexplained flu-like illness or acute bronchopneumonia should be tested for novel influenza A (H1N1), especially if there is a history of contact with known cases or with children with flu-like symptoms.

For pathologists, these cases highlight several important points. First, they illustrate the fact that DAD is a major histologic manifestation of viral infection. Other viral infections associated with DAD include avian influenza (H5N1),[23] severe acute respiratory syndrome coronavirus,[24] adenovirus, hantavirus, herpesvirus, cytomegalovirus, parainfluenza virus, and measles virus. Noninfectious causes of DAD also need to be considered, including drug reactions, toxic inhalants, gastric acid aspiration, and connective tissue disease.[25] In the vast majority of cases, the histologic features of DAD are nonspecific and do not allow determination of a precise cause. Therefore, results of ancillary testing, as in our cases, are often pivotal in establishing a specific etiologic agent.

Second, histologic clues classically associated with certain etiologic agents may not always be present. For example, necrotizing bronchitis/bronchiolitis and squamous metaplasia are thought to be classic features of influenza pneumonia. However, neither feature was prominent in our cases. Is the absence of these features peculiar to novel influenza A (H1N1) infection or is it simply a reflection of the histologic heterogeneity of influenza virus infection from case to case? The answer to this question will require study of larger numbers of cases.

Finally, our second case demonstrates that acute bacterial bronchopneumonia may coexist with, or mask, an underlying process. Pathologists must be aware that the presence of acute bronchopneumonia does not preclude an underlying viral infection. Careful attention to histologic abnormalities in lung parenchyma distant from the areas of bronchopneumonia may be helpful in this regard.

Novel influenza A (H1N1) virus infection may be encountered in widely disparate clinical settings and may result in strikingly dissimilar pathologic findings. The diagnosis should be suspected clinically in patients with an unexplained flu-like illness or acute pneumonia. Pathologists should add novel influenza A (H1N1) virus infection to the list of causes of DAD. Classic findings of influenza pneumonia, such as necrotizing bronchitis/bronchiolitis and squamous metaplasia, may not be prominent. A superimposed acute bacterial bronchopneumonia may mask underlying virus-related histologic changes. The diagnosis can be confirmed by rRT-PCR on specimens obtained at autopsy.

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