Myocardial Injury and Bacterial Pneumonia Contribute to the Pathogenesis of Fatal Influenza B Virus Infection

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(See the editorial commentary by McCullers and Hayden, on pages 870–2.)

Background. Influenza B virus infection causes rates of hospitalization and influenza-associated pneumonia similar to seasonal influenza A virus infection and accounts for a substantial percentage of all influenza-related hospitalizations and deaths among those aged <18 years; however, the pathogenesis of fatal influenza B virus infection is poorly described.

Methods. Tissue samples obtained at autopsy from 45 case patients with fatal influenza B virus infection were evaluated by light microscopy and immunohistochemical assays for influenza B virus, various bacterial pathogens, and complement components C4d and C9, to identify the cellular tropism of influenza B virus, characterize concomitant bacterial pneumonia, and describe the spectrum of cardiopulmonary injury.

Results. Viral antigens were localized to ciliated respiratory epithelium and cells of submucosal glands and ducts. Concomitant bacterial pneumonia, caused predominantly by Staphylococcus aureus, was identified in 38% of case patients and occurred with significantly greater frequency in those aged >18 years. Pathologic evidence of myocardial injury was identified in 69% of case patients for whom cardiac tissue samples were available for examination, predominantly in case patients aged <18 years.

Conclusions. Our findings suggest that bacterial pneumonia and cardiac injury contribute to fatal outcomes after infection with influenza B virus and that the frequency of these manifestations may be age related.

Precisely because influenza A is so important, the significance of influenza B is apt to be underestimated.1

WB Baine, JP Luby, and SM Martin

Seasonal influenza is caused by 2 groups of antigenically, ecologically, and epidemiologically distinct viruses in the family Orthomyxoviridae that cocirculate among human populations. Influenza A viruses garner the greatest attention because of the ability to undergo unpredictable antigenic changes in the major surface glycoproteins that contribute to annual epidemics among susceptible populations in temperate climates. Influenza A viruses also infect and can undergo genetic reassortment in a broad range of avian and mammalian hosts. Periodically, the emergence and sustained transmission of a novel influenza A virus subtype among human populations has created some of the most devastating and explosive infectious disease pandemics of the past 100 years [2, 3].

By comparison, influenza B viruses do not undergo antigenic shift or cause pandemics, and antigenic drift occurs more slowly than in influenza A viruses. Only 2 distinct lineages of influenza B viruses, comprising the Victoria-like and Yamagata-like strains, currently circulate among human populations [4], and the only known wildlife hosts of influenza B viruses are seals [5]. Influenza B viruses seldom predominate during seasonal
influenza epidemics, but when this occurs, mortality statistics are generally intermediate to those attributable to the influenza A virus subtypes [6–9]. Historical and contemporary descriptions of influenza pathology have characteristically focused, predominantly or exclusively, on disease caused by seasonal or pandemic influenza A viruses [2, 3, 10–15].

To our knowledge, there are no comprehensive studies to describe the pathology of influenza B virus infection in a large series of patients with fatal disease or to distinguish the pathology of disease with and without concomitant bacterial pneumonia. To better understand the pathogenesis of fatal influenza B virus infection, we evaluated the histological and immunohistochemical characteristics of autopsy tissue specimens collected from a large series of patients with confirmed influenza B and examined the frequency and composition of concomitant bacterial pneumonia, the cellular tropism of the virus, and the pathologic spectrum of cardiopulmonary injury.

METHODS

Patients

Tissue samples were obtained at autopsy from patients for whom influenza or another infectious cause of death was suspected on the basis of compatible clinical features of the illness and submitted subsequently to the Centers for Disease Control and Prevention (CDC) for diagnostic evaluation during May 2000–February 2010. Patients were identified by medical examiners, coroners, community-based pathologists, infectious diseases clinicians, and state and local health department personnel. Fatal pediatric influenza became a nationally notifiable condition in the United States in 2004 [16], and the quality and consistency of supplementary clinical and epidemiologic data were generally better for those patients evaluated during 2004–2010 than for those evaluated during 2000–2003.

Histopathology and Immunohistochemistry

Histological features were assessed from representative sections of airway, lung, cardiac, and lymph node tissues and tested for evidence of infection with influenza B virus using an immunoalkaline phosphatase technique. The primary antibodies were diluted at 1:2000 and consisted of pooled monoclonal immunoglobulins, comprising clones B2 and B4, that react with the influenza B virus nucleocapsid and hemagglutinin, respectively [17]. Its specificity was assessed by evaluating its reactivity with formalin-fixed, paraffin-embedded Madin-Darby canine kidney cells infected with influenza B virus strains Beijing/243/97 and HK/16/03 and with influenza A virus subtypes H1N1, H3N2, and H5N1.

Lung tissue samples from each case patient with intraalveolar inflammatory cell infiltrates were examined for bacterial coinfection by using Brown and Brenn or Lillie-Twort tissue Gram stains and Steiner or Warthin-Starry silver techniques. Lung sections from all patients with intraleukocytic bacteria identified by using these methods were evaluated by using immunohistochemical (IHC) assays to detect antigens of Streptococcus pneumoniae, Streptococcus pyogenes, Staphylococcus aureus, Haemophilus influenzae, Legionella pneumophila, group B Streptococcus, or Neisseria meningitidis [14, 15].

Cardiac tissue samples were examined for acute myocyte injury by routine histology and IHC assays for various complement components [18], using a polyclonal anti-human C4d antibody (American Research Products) diluted at 1:50 and a monoclonal anti-human C9 antibody (Leica Microsystems) diluted at 1:200. A cardiac tissue sample from a patient with Coxsackie B1 viral myocarditis was used as a positive control. Negative controls included cardiac tissues with no histologic evidence of inflammation or myocyte injury, obtained at autopsy from 8 patients who died suddenly of a noninfectious cause and from 15 patients who died of confirmed viral or bacterial infections other than influenza B virus infection.

Molecular Analyses

RNA was extracted from formalin-fixed, paraffin-embedded tissue samples and evaluated by using a real-time reverse-transcriptase polymerase chain reaction (rRT-PCR) assay for the presence of influenza B virus hemagglutinin gene segments [19]. Lineage-specific BHQplus probes (Biosearch Technologies) were used to discriminate the hemagglutinin genes of currently circulating Victoria-like and Yamagata-like influenza B viruses [20]. The rRT-PCRs were performed using the following parameters: 50°C for 30 minutes, 95°C for 2 minutes, and 45 cycles of 95°C for 15 sec and 55°C for 30 sec. From the formalin-fixed, paraffin-embedded tissue blocks that showed immunostaining for S. aureus, DNA was extracted as described elsewhere [15] and used as template for 2 multiplex real-time PCR assays targeting segments of the muc (heat-stable DNA nuclease) and PVL (Panton-Valentine leukocidin) genes and mecA (a methicillin-resistance determinant) gene [21, 22]. For all real time assays, Ct values ≤40 were considered to be positive.

Statistical Analyses

Selected features were compared between the groups of case patients with and without concomitant bacterial pneumonia and between case patients with and without evidence of myocardial injury. Categorical features were compared between the 2 groups using the 2-sided Fisher’s exact test. Continuous data were compared between groups using the Wilcoxon rank-sum test. A P value of < .05 was considered to be statistically significant.

RESULTS

Patient Characteristics

Tissue samples from 45 case patients (29 female and 16 male patients) with influenza B virus infection were examined
Table 1. Comparison of Selected Demographic, Clinical, and Laboratory Characteristics of Case Patients With Fatal Influenza B Virus Infection, With or Without Pathologic Evidence of Bacterial Pneumonia, Evaluated at the Centers for Disease Control and Prevention (CDC), 2000–2010

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients With No Pathologic Evidence of Bacterial Pneumonia (n = 28)</th>
<th>Patients With Pathologic Evidence of Bacterial Pneumoniaa (n = 17)</th>
<th>Number (%)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. females</td>
<td>19 (68)</td>
<td>10 (59)</td>
<td></td>
</tr>
<tr>
<td>Median years-of-age (range)c</td>
<td>6.5 (1–34)</td>
<td>20 (0–55)</td>
<td></td>
</tr>
<tr>
<td>No. &lt;18 years of agec</td>
<td>26 (93)</td>
<td>8 (47)</td>
<td></td>
</tr>
<tr>
<td>No. with ≥1 high-risk conditiond</td>
<td>9 (43)</td>
<td>4 (33)</td>
<td></td>
</tr>
<tr>
<td>Median days from illness onset to death (range)</td>
<td>3 (1–22)</td>
<td>4 (2–8)</td>
<td></td>
</tr>
<tr>
<td>No. who died at home or en route to hospital</td>
<td>9 (35)</td>
<td>5 (31)</td>
<td></td>
</tr>
<tr>
<td>No. who died at a clinic or emergency department</td>
<td>6 (23)</td>
<td>3 (19)</td>
<td></td>
</tr>
<tr>
<td>No. hospitalized</td>
<td>11 (42)</td>
<td>8 (50)</td>
<td></td>
</tr>
<tr>
<td>Confirmation of influenza B virus infection outside of CDC</td>
<td>23 (82)</td>
<td>13 (81)</td>
<td></td>
</tr>
<tr>
<td>Culture isolation</td>
<td>14 (61)</td>
<td>6 (43)</td>
<td></td>
</tr>
<tr>
<td>Rapid antigen detection</td>
<td>4 (17)</td>
<td>5 (36)</td>
<td></td>
</tr>
<tr>
<td>Direct fluorescent antibody</td>
<td>2 (9)</td>
<td>1 (7)</td>
<td></td>
</tr>
<tr>
<td>RT-PCR</td>
<td>9 (39)</td>
<td>2 (15)</td>
<td></td>
</tr>
<tr>
<td>Confirmation of influenza B virus infection at CDC</td>
<td>28 (100)</td>
<td>17 (100)</td>
<td></td>
</tr>
<tr>
<td>RT-PCR</td>
<td>16 (59)</td>
<td>9 (60)</td>
<td></td>
</tr>
<tr>
<td>Victoria-like</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yamagata-like</td>
<td>11 (41)</td>
<td>6 (40)</td>
<td></td>
</tr>
<tr>
<td>Immunohistochemistryc</td>
<td>22 (79)</td>
<td>6 (35)</td>
<td></td>
</tr>
</tbody>
</table>

a Bacterial pneumonia confirmed by histopathology, tissue Gram and silver techniques, immunohistochemical stains, and PCR assays; comprising Staphylococcus aureus (13 patients), Streptococcus pyogenes (1), group B Streptococcus (Streptococcus agalactiae (1), Neisseria meningitidis (1), and undetermined (1).  

b Number in parentheses represents percentage expressed as the number of patients with specific characteristic/the number of patients with data available for the characteristic, unless otherwise specified.  

c Significance between groups, P < .001.  

d Preexisting high-risk conditions, other than young age, included asthma (4 patients); diabetes mellitus (3); leukemia (2); seizure disorder (2); cerebral palsy (1); ventricular septal defect (1), and pregnancy (1). Two patients had 2 of these conditions.

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Laboratory Confirmation of Influenza B Virus Infection

Infection with influenza B virus was identified in 36 (82%) of the case patients by at least 1 assay before submission to the CDC. At the CDC, influenza B viral nucleic acids were detected in formalin-fixed, paraffin-embedded samples of respiratory tissue from all case patients; of the influenza B viruses that could be characterized, 25 were Victoria-like and 17 Yamagata-like strains (Table 1). Only Yamagata-like strains were identified from 2008 (n = 11), whereas Victoria-like strains were identified in all tissues from case patients from 2003 (n = 4), 2007 (n = 9), and 2009 (n = 9). All other years were represented by 1 influenza B virus lineage or a combination of Yamagata-like and Victoria-like strains. Viral antigens were detected by IHC staining of respiratory tissues from 28 case patients. Tissue samples from case-patients without bacterial pneumonia were more likely to be IHC positive for viral antigens than those from case patients with evidence of bacterial coinfection (P < .001) (Table 1). When case patients without a concomitant bacterial pneumonia were stratified by age group, those aged <18 years were more likely to be...
IHC-positive for influenza B virus than were those aged \( \geq 18 \) years \((P = .03)\).

**Pulmonary Pathology**

The predominant pathologic feature common to almost all case patients was inflammation of the trachea and bronchi (Table 2; Figure 1A). The epithelial surfaces of these airways were often denuded and associated with necrosis and hemorrhage; however, in most cases, the basal layer of epithelium and basement membrane were preserved (Figure 1B). Inflammatory cell infiltrates were characterized predominantly by lymphocytes, plasma cells, and macrophages that infiltrated the lamina propria and often extended into the submucosa to involve seromucinous glands and ciliated ducts (Figure 1B). Close inspection of glandular epithelium occasionally revealed focal necrosis. Marked congestion of mural capillaries and edema of adjacent connective tissues were conspicuous and common features. Bronchiolitis was observed in \( \sim 40\% \) of case patients without a superimposed bacterial infection (Figure 1C).

Erythrophagocytosis was identified in lymph nodes adjacent to trachea and bronchi of 10 (34%) of the 29 case patients for whom these tissues were available (Figure 1D).

For 17 (38%) case patients, intraalveolar collections of neutrophils and macrophages containing intracellular bacteria were identified by using tissue Gram or silver techniques (Table 1). IHC and PCR assays confirmed coinfection with *S. aureus* in 13 (76%) of the patients with bacterial pneumonia, of whom 7 had genetic markers for methicillin resistance and 11 for Panton-Valentine leukocidin. All of the case patients with pneumonia due to infection with *S. aureus* and 1 due to *S. pyogenes* showed diffusely necrotizing pulmonary lesions, accompanied by abundant hemorrhage and edema, distinguished by coalesced areas of necrosis and hemorrhage that destroyed the normal architecture of the lungs (Figure 1E). Other pulmonary lesions, including intraalveolar hemorrhage and edema, interstitial pneumonia (Figure 1F), and alveolitis were observed in \( \leq 50\% \) of case patients without evidence of bacterial pneumonia (Table 2).

**Distribution of Viral Antigens**

Antigens of influenza B virus were detected predominantly in the nuclei of ciliated columnar epithelium of the trachea and proximal bronchi and less frequently in cells lining the bronchioles (Table 2; Figure 2A and 2B). Antigens were also identified in the epithelium of submucosal glands and ciliated ducts of the trachea and bronchi (Figure 2C and 2D) and uncommonly in airspaces, comprising predominantly detached strips of ciliated airway epithelium, or single cells, situated freely within the alveoli. Viral antigens were detected infrequently in respiratory tissues from most IHC-positive

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**Table 2. Histological and Immunohistochemical Features of Case Patients With Fatal Influenza B Virus Infection, With or Without Pathologic Evidence of Bacterial Pneumonia, Evaluated at the Centers for Disease Control and Prevention, 2000–2010**

<table>
<thead>
<tr>
<th>Histological or Immunohistochemical Feature</th>
<th>Patients With No Pathologic Evidence of Bacterial Pneumonia (n = 28)</th>
<th>Patients With Pathologic Evidence of Bacterial Pneumoniaa (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tracheitis</td>
<td>20 (100)</td>
<td>11 (100)</td>
</tr>
<tr>
<td>Bronchitis</td>
<td>23 (96)</td>
<td>14 (100)</td>
</tr>
<tr>
<td>Bronchiolitisc</td>
<td>11 (39)</td>
<td>13 (76)</td>
</tr>
<tr>
<td>Pulmonary hemorrhagea</td>
<td>14 (50)</td>
<td>16 (94)</td>
</tr>
<tr>
<td>Pulmonary edema</td>
<td>8 (29)</td>
<td>12 (70)</td>
</tr>
<tr>
<td>Interstitial pneumonia</td>
<td>6 (21)</td>
<td>1 (6)</td>
</tr>
<tr>
<td>Alveolitisa</td>
<td>5 (18)</td>
<td>1 (6)</td>
</tr>
<tr>
<td>Diffuse alveolar damage</td>
<td>5 (18)</td>
<td>3 (18)</td>
</tr>
<tr>
<td>Erythrophagocytosis in airway lymph nodes</td>
<td>8 (44)</td>
<td>2 (18)</td>
</tr>
</tbody>
</table>

IHC distribution of viral antigens

<table>
<thead>
<tr>
<th></th>
<th>Patients With No Pathologic Evidence of Bacterial Pneumonia (n = 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tracheia</td>
<td>9 (47)</td>
</tr>
<tr>
<td>Bronchies</td>
<td>13 (57)</td>
</tr>
<tr>
<td>Bronchioles</td>
<td>8 (29)</td>
</tr>
<tr>
<td>Submucosal glands</td>
<td>8 (31)</td>
</tr>
<tr>
<td>Ciliated ductsc</td>
<td>13 (50)</td>
</tr>
<tr>
<td>Alveoli</td>
<td>5 (18)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Patients With Pathologic Evidence of Bacterial Pneumoniaa (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tracheia</td>
<td>1 (9)</td>
</tr>
<tr>
<td>Bronchies</td>
<td>1 (14)</td>
</tr>
<tr>
<td>Bronchioles</td>
<td>4 (24)</td>
</tr>
<tr>
<td>Submucosal glands</td>
<td>2 (14)</td>
</tr>
<tr>
<td>Ciliated ductsc</td>
<td>2 (14)</td>
</tr>
<tr>
<td>Alveoli</td>
<td>2 (12)</td>
</tr>
</tbody>
</table>

a *Staphylococcus aureus* (13 patients), *Streptococcus pyogenes* (1), group B *Streptococcus* (1), *Neisseria meningitidis* (1) and undetermined (1).

b Number in parentheses represents percentage expressed as the number of patients with the specific characteristic/the number of patients with representative tissue for evaluation of the characteristic.

c Significance between groups, \( P < .05 \).

d Significance between groups, \( P < .01 \).
patients, and extensive staining was relatively unusual. Of case patients for whom antigens could be detected and a duration of illness was available, 13 (72%) died within 4 days after illness onset and only 2 (11%) survived for >7 days, including a 13-year-old child with acute myeloblastic leukemia in whom viral antigens were detected by IHC at 22 days.

**Cardiac Pathology**

Myocardial injury was identified by histological and IHC assessment in 20 (69%) of 29 case patients with cardiac tissue samples available. For 17 case patients, there was histological evidence of myocyte damage, including 10 with unequivocal myocarditis, characterized by multiple discrete foci of myocyte necrosis accompanied by clusters of interstitial lymphocytes, macrophages, and occasional neutrophils (Figure 3A). Histological evidence of myocardial injury was characterized in 1 case patient by a small acute myocardial infarct and, in 6 others, by variably sized foci of shrunken or hypereosinophilic myocytes lacking cross-striations and nuclei (Figure 3B). Of 28 case patients for whom heart tissue samples were available to detect C4d and C9 by IHC, 19 revealed multifocal cytoplasmic staining of contiguous intramural myocytes that stained reproducibly with both of the anticomplement antibodies. This pattern of staining was observed in the myocardium of each case patient.

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**Figure 1.** Histopathology of fatal influenza B virus infection. A, Trachea of a 10-year-old girl with focally denuded epithelium and extensive mononuclear inflammatory cell infiltrates and prominent vascular congestion in the lamina propria. B, Lymphocytic infiltrates involving a submucosal gland and a ciliated duct in the bronchus of a 17-year-old girl. C, Inflamed and congested bronchiole with transmural infiltrates and damaged epithelium in the lung of a 5-year-old girl. D, Red cells phagocytosed by sinusoidal macrophages in a parabronchial lymph node of a 10-year-old boy. E, Extensive necrosis and colonies of *Staphylococcus aureus* bacteria in the lung of a 51-year-old man. F, Interstitial pneumonia in a 2-year-old girl. Hematoxylin and eosin stains; original magnifications ×25 (A, B, F), ×12.5 (C), ×100 (D), and ×50 (E).
with abnormal histological findings for whom tissues remained for testing (Figures 3C–3F) and in cardiac tissues of 3 other case patients lacking specific histological evidence of myocyte injury. When patients with histological or IHC evidence of cardiac injury were compared with those without this evidence by various demographic, laboratory, and clinical features, only the frequency of bacterial pneumonia was identified as statistically significant ($P < .01$) (Table 3).

No viral antigens were detected by IHC stain in the myocardium of any patient. Conspicuous staining of C4d or C9 was not identified in any of the cardiac tissues from the 8 patients with sudden traumatic death. Among control tissues obtained from 15 patients who died of an infectious process other than influenza B virus infection, focal staining of C4d and C9 was observed only in rare cardiac myocytes of a 6-year-old child with enteroviral meningoencephalitis and a 3-year-old child with pneumococcal pneumonia. Nucleic acids of influenza B virus were amplified by rRT-PCR from formalin-fixed, paraffin-embedded myocardium from only 1 case patient.

**DISCUSSION**

This study revealed several important features associated with fatal influenza B virus infection, including the rapidity with which this disease can culminate in death, the histological similarity with fatal seasonal influenza A virus infection, the relative infrequency of concomitant bacterial pneumonia in young patients, and the frequency of cardiac injury. Indeed, cardiac injury and bacterial pneumonia were prominent findings in this cohort, and one or both features were identified in $\sim$90% of the case patients for whom these pathologic characteristics could be assessed.

For many years, clinicians and epidemiologists considered influenza B to be a disease of milder severity than seasonal
influenza A [23]; however, contemporary studies indicate that influenza B is clinically indistinguishable from seasonal influenza A, causes similar rates of hospitalization and influenza-associated pneumonia, and accounts for a substantial percentage of all influenza-related hospitalizations and deaths in patients aged <18 years [6, 8, 24–27]. Of note, 34% of the 309 seasonal influenza-associated deaths in children reported to the CDC during 2004–2008 were attributed to influenza B [16] (CDC, unpublished data), and many of the case patients identified in our series had no recognized risk factor for severe influenza other than young age. Fatal influenza B virus infection occurs most frequently among pediatric patients; however, influenza B is also associated with lethal outcomes and substantial seasonal morbidity among adults [28, 29].

For most case patients in this series, death followed a remarkably rapid clinical progression, regardless of demonstrable bacterial pneumonia: 50% of all case patients died within 3 days after illness onset, and ~70% died within 4 days. The time from onset to death observed with fatal influenza B was more rapid than the duration of fatal illness described for pandemic 1918 A(H1N1), pandemic 1957 A(H2N2), pandemic 1968 A(H3N2), pandemic 2009 H1N1, and seasonal A(H3N2) viruses [3, 10–13, 28, 29].

Figure 3. Histopathology and immunohistochemical staining of cardiac injury in patients with fatal influenza B virus infection. A, Myocarditis in a 2-year-old girl showing mixed interstitial inflammatory cell infiltrates and multifocal myocyte necrosis. B, Focus of myocyte injury in a 9-year-old girl showing muscle cells with flocculated cytoplasm lacking cross-striations and nuclei. C and D, Immunohistochemical staining (red) for complement components C9 (C) and C4d (D) in a focus of inflamed myocardium in a 10-year-old boy. E and F, Staining of C4d in another focus lacking inflammatory infiltrates. Damaged myocytes show hypereosinophilic and coagulated cytoplasm and nuclear loss. Hematoxylin and eosin stains (A, B, F) and immunohistochemistry for complement C9 (C) and C4d (D) original magnifications ×25 (A), ×50 (B, C, D), and ×158 (E, F).
The pathogenic basis for these variations remains to be determined, particularly with respect to disease solely due to an influenza virus, and various biases, including age structure, bacterial pneumonia, or unrecognized or unreported risk factors, could influence these findings.

Bacterial pneumonia in this cohort of case patients with fatal influenza B was represented predominantly by *S. aureus*, which has been the most commonly identified etiology among US hospitalized pediatric patients with influenza-associated bacterial pneumonia since the 2003–2004 influenza season [16, 32]. Among the adult case patients with influenza B virus infection, fatal disease occurred significantly more often in the context of a combined bacterial pneumonia: 82% of patients aged \( \geq 18 \) years died with evidence of bacterial pneumonia, compared with only 24% of those patients \( < 18 \) years of age. Because influenza B is often considered to be a relatively mild illness, many clinicians have ascribed lethal disease solely to concomitant bacterial pneumonia. As recently as 1973, Maxwell Finland, a highly regarded infectious diseases physician who had studied influenza B for many decades, wrote that he had, “not yet authenticated any fatal case of influenza B viral pneumonia in which there was not a prominent bacterial component” [33, p 96]. Nonetheless, our findings and others [24, 34] indicate that fatal disease in children can occur in the absence of concomitant bacterial pneumonia.

We identified histological or IHC evidence of myocardial injury in 69% of the case patients in this series for whom cardiac tissue samples were available for evaluation. Histological features of myocarditis were apparent in 50% of these patients; of surprise, the cardiac tissue samples from the other 50% demonstrated multifocal IHC evidence of damage that was histologically compatible with ischemic necrosis in the absence of conspicuous inflammatory cell infiltrates. To determine whether these changes might be associated with postmortem artifact or a generalized observation associated with the terminal stage of any of several infectious disease processes other

### Table 3

**Selected Features of Case Patients With Fatal Influenza B Virus Infection, With or Without Pathologic Evidence of Cardiac Injury, Evaluated at the Centers for Disease Control and Prevention, 2000–2010**

<table>
<thead>
<tr>
<th>Clinical, Demographic, or Laboratory Feature</th>
<th>Patients With Cardiac Injury(^a) (n = 20)</th>
<th>Patients Without Cardiac Injury (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median years of age (range)</td>
<td>10 (0–34)</td>
<td>14 (0–55)</td>
</tr>
<tr>
<td>No. females</td>
<td>13 (65)</td>
<td>5 (56)</td>
</tr>
<tr>
<td>No. infected with a Victoria-like strain</td>
<td>12 (67)</td>
<td>4 (50)</td>
</tr>
<tr>
<td>Median days from onset of illness to death (range)</td>
<td>4 (2–10)</td>
<td>3 (1–8)</td>
</tr>
<tr>
<td>No. with bacterial pneumonia(^a)</td>
<td>3 (15)</td>
<td>6 (67)</td>
</tr>
</tbody>
</table>

\(^a\) Includes histologic evidence of myocarditis or multifocal immunohistochemical staining of C4d and C9.

\(^b\) Number in parentheses represents percentage expressed as the number of patients with the specific characteristic/the number of patients with representative tissue for evaluation of the characteristic.

\(^c\) Significance between groups, \( P < .01 \).
than influenza B, we evaluated cardiac tissues from cohorts of patients who died of sudden noninfectious causes and of multiple infectious etiologies other than influenza B. The frequency of myocardial injury among the case patients with influenza B without histological evidence of myocarditis was statistically greater than observed among the patients who died of other infectious causes \( (P = 0.03) \), suggesting that cardiac injury, directly or indirectly associated with influenza B virus infection, contributed prominently to the deaths of many of the case patients who we studied. Indeed, damage to cardiac muscle was evident in 85% of patients with myocardium for evaluation who did not have bacterial pneumonia.

Myocarditis or myocardial necrosis without inflammatory cell infiltrates have been described sporadically in patients with severe influenza B [35–37], and there are a few reports of sudden unexpected cardiac arrest or rapidly developing manifestations suggestive of myocardial ischemia in previously healthy children infected with influenza B virus [38, 39]; however, we are not aware of any large studies that specifically identify cardiac injury as a frequent manifestation of fatal influenza B. Skeletal myopathy is described in 14%–48% of children infected with influenza B virus [26, 40, 41], and many of these patients also present with elevated levels of cardiac muscle creatine phosphokinase [42]. Histological descriptions of influenza B–associated skeletal myopathy are remarkably similar to the cardiac pathology identified in several of the case patients in our series and include randomly distributed foci of segmental hyaline degeneration and necrosis of individual fibers, often in the absence of any inflammatory infiltrates [43, 44]. The pathogenic mechanisms that lead to injury to striated fibers in the heart and skeletal muscles remain unknown. Influenza B virus can replicate in regenerating human muscle cell cultures, and it has been suggested that myogenic events associated with addition of new muscle fibers during muscle growth may render children more susceptible to influenza-associated myopathy than older patients, in whom regenerative myogenic events are less frequent [45]; indeed, 90% of the case patients in this series with myocardial injury were aged <18 years.

IHC stains for C4d and C9 have been used to identify early myocardial infarcts in patients who die before an influx of leukocytes can occur [18]. In our evaluation, we identified a reproducible pattern of staining with both antibodies in cardiac tissues from the majority of case patients with fatal influenza B virus infection; however, no viral antigens were identified in the damaged myocardium of any case patient, and viral nucleic acids were detected in the cardiac tissue from only one patient. Myocarditis is also reported occasionally among patients with fatal influenza A virus infection [2, 10, 14], and further study is needed to better determine the relative contributions of viral-mediated injury and ischemia to cardiac tissues of severely ill patients during seasonal influenza epidemics.

The few studies to describe in vivo distribution of influenza virus antigens in respiratory tissues from deceased patients have focused specifically on influenza A viruses [14, 15]. Preferential binding of influenza B viruses to receptors represented by sialic acid connected to galactose by a 2, 6 linkage (SAα2, 6Gal) has been identified in vitro and is predicted from a structural analysis of the influenza B hemagglutinin molecule [46]. We identified a consistent pattern of IHC staining in the ciliated epithelium of the airways of case patients that closely approximated the cellular tropism exhibited in respiratory tissues from patients infected with seasonal human-adapted influenza A viruses, which also bind preferentially to (SAα2, 6Gal) receptors; in this context, our data support the hypothesis that (SAα2, 6Gal) is the preferred receptor for influenza B virus. Influenza B virus also infects acinar cells of the submucosal glands in the trachea and bronchi, similar to seasonal A (H3N2) and pandemic A (H1N1) viruses [14, 15]. Viral-mediated injury to these cells and to the epithelium lining large airways and ciliated ducts undoubtedly contributes to the evolution of influenza-associated bacterial pneumonia by compromising the mucus stream and hindering mucociliary clearance of bacteria from the respiratory tract [47, 48].

For decades, the public health impact of influenza B virus has been overshadowed by the magnitude of disease caused by influenza A viruses; however, the infectious burden of influenza B virus should not be underestimated: of the 115 pediatric influenza-associated deaths in the United States reported to the CDC during the 2010–2011 influenza season, 44 (38%) involved influenza B virus infection [49], and the life-threatening consequences exacted by coinfections with influenza B virus and S. aureus or S. pyogenes are increasingly described [16, 29, 50]. Our study was limited by its restricted analysis of case patients for whom an autopsy was performed and tissue samples sent to the CDC and by qualitative and quantitative differences among case patient tissue samples submitted for evaluation. Despite these limitations, our findings suggest that increased awareness of potentially fatal outcomes in patients infected with influenza B virus is needed from the clinical and public health community to develop prevention and control strategies commensurate with the importance of this pathogen.

**Notes**

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