MAJOR ARTICLE

Histopathologic and Immunohistochemical Features of Fatal Influenza Virus Infection in Children during the 2003–2004 Season

Jeannette Guarner,[1] Christopher D. Paddock,[1] Wun-Ju Shieh,[1] Michelle M. Packard,[1] Mitesh Patel,[1] Jeltley L. Montague,[1] Timothy M. Uyeki,[2] Niranjan Bhat,[2] Amanda Balish,[2] Stephen Lindstrom,[2] Alexander Klimov,[2] and Sherif R. Zaki[1]

[1]Infectious Disease Pathology Activity and [2]Influenza Branch, Division of Viral Rickettsial Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia

Background. The Centers for Disease Control and Prevention enhanced national surveillance for influenza-associated deaths among children because of early reports of pediatric deaths during the 2003–2004 influenza season.

Methods. We studied lung and upper airway specimens from 47 case patients who died who had at least 1 positive result for influenza virus tests using hematoxylin and eosin, special stains for bacteria and fungi, and immunohistochemical (IHC) assays for influenza A and B viruses and other potential viral and bacterial respiratory pathogens.

Results. Nineteen (40%) of the 47 patients were ⩽2 years old, and 26 (55%) were female. Influenza IHC testing identified type A antigens in 26 patients and type B antigens in 1 patient. Influenza antigens were observed focally in bronchoepithelial cells and mucous glands of trachea, bronchi, and larger bronchioli, showing submucosal mononuclear inflammatory infiltrates. IHC assays were the only confirmatory diagnostic test for 5 patients (11%). Significant life-threatening pathological conditions that could be considered the cause of death were present in 36 patients (77%) and included diffuse alveolar damage (12 cases), extensive secondary pneumonia (11 cases), extensive intraalveolar hemorrhage (10 cases), viral pneumonitis (10 cases), myocarditis (6 cases), and meningoencephalitis (1 case). For 9 patients with bronchopneumonia, a bacterial or fungal etiology was determined with IHC assay (3 *Staphylococcus* infections, 3 group A streptococci infections, 1 *Streptococcus pneumoniae* infection, 1 *Bordetella pertussis* infection, and 1 *Aspergillus* infection).

Conclusions. IHC assays are useful for the diagnosis of influenza and bacterial pneumonia. This study underscores the importance of performing autopsies to identify the causes of death in patients with influenza infection.

Every year in the United States, influenza causes substantial morbidity and mortality, particularly among infants, adults with chronic cardiopulmonary disease, and elderly persons [1]. In response to early reports of influenza-associated pediatric deaths during the 2003–2004 season, the Centers for Disease Control and Prevention (CDC) implemented enhanced surveillance for possible influenza-associated deaths among children. At the time, influenza-associated deaths were not a nationally reportable condition [2–5]. As a result of the enhanced surveillance, 153 pediatric deaths associated with influenza were reported from 40 states during the 2003–2004 influenza season [2, 5]. For many of these patients, the only tissue samples available for diagnostic testing were formalin-fixed, paraffin-embedded specimens that had been obtained at autopsy.

Most published pathology reports of fatal influenza virus infection relate to pandemic cases, and findings include necrotizing bronchitis, thrombosis, interstitial inflammation, and hyaline membrane formation, accompanied by various degrees of intraalveolar edema, hemorrhage, and inflammation [6–11]. In contrast to other viral infections, influenza does not produce a viral cytopathic effect in histopathologic preparations. Sec-

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The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

Reprints or correspondence: Dr. Jeannette Guarner, Infectious Disease Pathology Activity, Centers for Disease Control and Prevention, Mailstop G32, 1600 Clifton Rd. NE, Atlanta GA 30333 (jguarner@cdc.gov).

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ondary bacterial pneumonia may occur as a complication and make it difficult to distinguish the pathologic changes associated with primary influenza virus infection [8, 12]. A previous immunohistochemical (IHC) study of lung samples from 8 patients who died of influenza A demonstrated influenza antigens in few bronchioepithelial cells of larger bronchioli [12].

Here, we describe the histopathologic changes in formalin-fixed, paraffin-embedded tissue specimens obtained from children with fatal influenza during the 2003–2004 season and the application of IHC assays for the detection and localization of influenza virus antigens. To identify the pathologic conditions played by secondary infections, special stains and IHC assays for selected bacteria, fungi, and viruses were applied to lung specimens from these patients.

MATERIALS AND METHODS

From December 2003 to July 2004, the CDC requested postmortem tissue specimens from 91 children <18 years old who were suspected to have had influenza virus infection or who had died of an unexplained illness. Available demographic data, results of laboratory testing for influenza and bacteria performed as part of the patients' case management, and relevant clinical information were collected. Only patients with at least 1 positive laboratory test result for influenza (i.e., results of rapid antigen testing, direct fluorescent antibody assay, EIA, RT-PCR, culture, and/or IHC assay) were included in this study. For the patients studied, hematoxylin-eosin stains of specimens from the major airways and lung were further evaluated for presence of inflammation, hemorrhage, edema, and cell necrosis. Available heart or CNS specimens were evaluated for myocarditis or encephalitis. In the lymph nodes or other lymphoid organs, the presence of hemophagocytosis was evaluated.

Gram and Steiner silver impregnation stains were used to study 1 block from upper airway specimens and 1 block from lung specimens, showing the most amount of inflammation for all cases. Lung blocks with evidence of pneumonia (defined as peribronchial and intraalveolar neutrophilic infiltrate) were examined for fungi with Grocott-Gomori methenamine–silver nitrate stains. IHC assays for influenza A and B viruses were performed on sections from all blocks with major airway or lung tissue specimens. IHC assays for adenoviruses and respiratory syncytial virus were performed on sections from the same blocks on which Gram and Steiner staining had been performed. IHC assays for *Streptococcus pneumoniae,* group A streptococci, *Staphylococcus aureus, Haemophilus influenzae, Neisseria meningitidis,* and *Klebsiella pneumoniae* were performed on sections from 2 lung tissue blocks with pneumonia. IHC assay for *Bordetella pertussis* was performed on available sections from 2 lung tissue blocks from all children ≤2 years old.

IHC assays. IHC assays for viruses and bacteria were performed as previously described [12–16]. After being deparaffinized and rehydrated, tissue sections were placed in a Dako autostainer (DakoCytomation) for digestion with proteinase K (Boehringer-Mannheim Corporation), blocked with normal sheep serum, and incubated for 1 h with the primary antibodies. Optimal dilutions for each primary antibody were determined in preliminary tests with formalin-fixed, paraffin-embedded control tissues (bacterial or viral cultures; table 1). Attached primary antibodies were detected by the LSAB2 universal alkaline phosphatase system (DakoCytomation) after which they were counterstained with Mayer's hematoxylin (Fisher Scientific).

All primary antibodies were prepared at the CDC (Atlanta, GA), except the antibodies against *S. aureus* (Biodesign), *N. meningitidis* (Center for Biologics Evaluation and Research, Food and Drug Administration), and *K. pneumoniae* (Biogenesis). Negative controls consisted of sequential case patient tissue sections incubated with normal rabbit, mouse, or horse serum as the primary antibody. Interpretation of IHC assay results for influenza virus included determination of (1) the location of the positive reaction in the cell (nuclear or cytoplasmic staining), (2) the type of cells infected (bronchoepithelial or glandular or cells present in the alveoli), and (3) the

Table 1. Antibodies used in immunohistochemical assays.

Antibody against pathogen	Source animal for serum	Dilution
Influenza A virus[a]	Mouse	1:1000
Influenza B virus[b]	Mouse	1:2000
Adenovirus	Mouse	1:2000
Respiratory syncytial virus	Mouse	1:1000
Streptococcus pneumoniae[c]	Rabbit	1:3000
GAS[d]	Rabbit	1:2000
Staphylococcus aureus[e]	Rabbit	1:500
Haemophilus influenzae[f]	Rabbit	1:10,000
Neisseria meningitidis[g]	Horse	1:500
Klebsiella pneumoniae[h]	Rabbit	1:200
Bordetella pertussis	Mouse	1:1000

NOTE. GAS, group A streptococci.

[a] The nuclear protein influenza A virus monoclonal antibody does not react with influenza B virus-infected cells.

[b] The nuclear protein influenza B virus monoclonal antibody does not react with influenza A virus-infected cells.

[c] The *S. pneumoniae* antibody does not react with GAS, group B streptococci (GBS), staphylococci, anthrax, tularemia, *Legionella* species, leptospirosis, *H. influenzae, Klebsiella* species, mycoplasma, mycobacteria, menigococi, or *Yersinia* species.

[d] The GAS antibody does not react with *S. pneumoniae*, GBS, staphylococci, anthrax, clostridia, listeria, *H. influenzae,* mycobacteria, menigococci, or rickettsiae.

[e] The *S. aureus* antibody does not react with GAS, GBS, *S. pneumoniae,* anthrax, clostridium, *Klebsiella* species, *Pseudomonas* species, or meningococci.

[f] The *H. influenzae* antibody does not react with GAS, GBS, *S. pneumoniae, Klebsiella* species, *Legionella* species, meningococci, or mycobacteria.

[g] The *N. meningitidis* antibody does not react with GAS, staphylococci, *Listeria* species, anthrax, tularemia, *H. influenzae,* mycoplasma, *Pseudomonas* species, or rickettsiae.

[h] The *K. pneumoniae* antibody does not react with GAS, GBS, *S. pneumoniae*, anthrax, *Clostridium* species, tularemia, *H. influenzae, Legionella* species, *Leptospira* species, meningococci, mycoplasma, or rickettsiae.

DFA	Rapid test	Culture	EIA	RT-PCR	IHC	Total number of patients with positive test results
	+				+	5
					+	5
+					+	3
	+	+	+		+	3
	+	+			+	3
+		+			+	2
+	+	+			+	2
	+		+		+	1
+	+		+		+	1
				+	+	1
			+		+	1
	+				-	6
				+	-	3
	+		+		-	2
			+		-	2
+		+			-	2
+					-	1
	+	+			-	2
+	+				-	1
	-		+		-	1

Figure 1. Diagnostic influenza test results for 47 pediatric patients who died of influenza. With the exception of immunohistochemical assay (IHC), RT-PCR, and some viral cultures, laboratory tests were available as part of the patient's care. There were 12 total positive results by direct fluorescent antibody assay (DFA), 27 by rapid test, 14 by culture, 11 by EIA, 4 by RT-PCR, and 27 by IHC. −, negative; +, positive.

amount of staining graded as rare (i.e., when occasional cells in a slide were stained) or as significant (i.e., when multiple cells were stained). Interpretation of results for bacterial IHC assays included evaluation of the staining pattern (well-defined bacteria vs. granular staining) and the location of the bacteria in relation to the pathologic features (e.g., inside inflammatory cells, necrotic material, and pleural inflammation).

RESULTS

A total of 47 patients had at least 1 positive influenza virus test result. Five patients (11%) were children aged <6 months, 8 (17%) were aged 6–23 months, 16 (34%) were aged 2–5 years, 11 (23%) were aged 6–12 years, and 7 (15%) were aged 13–17 years. There were 26 (55%) females. Figure 1 presents results of the different tests for influenza virus performed for the patients. Eighteen patients (38%) had positive results for influenza by 1 test only, including 6 patients with results from a rapid diagnostic test, 5 with results from IHC assay, 3 with results from RT-PCR, 3 with results from EIA, and 1 with a result from direct fluorescent antibody assay. Chronic medical conditions recognized by the Advisory Committee on Immunization Practices as factors that increase the risk of influenza-related complications were reported in 6 children (2 cases of asthma, 2 cases of other chronic pulmonary conditions, and 2 cases of metabolic disorder).

Table 2 presents the histopathologic features observed in major airways, lungs, and other organs. The most frequent histopathologic findings in major airways were congestion (in 36 [90%] of 40 patients), inflammation (29 [73%] of 40), necrosis of bronchial epithelium (20 [50%] of 40) (figure 2*A* and 2*B*), and hemorrhage (20 [50%] of 40) (figure 2*A*). The peribronchial inflammatory infiltrate contained >3 eosinophils per high power field (using a 40× magnification objective) in 40% of patients. In the lung samples, the most frequent findings were the presence of hyaline membranes (in 31 [67%] of 46 patients) and interstitial inflammation (31 [67%] of 46) (figure 2*C*). Hemophagocytosis was noted in 53% of paratracheal lymph nodes. Of 18 patients with available brain samples, 1 had multiple brain abscesses associated with hyphae in the meninges and brain parenchyma. Significant life-threatening pathologic conditions that could be considered the cause of death were present in 36 patients (77%). The conditions included diffuse alveolar damage (12 [26%] of 46 cases), extensive secondary pneumonia (11 [24%] of 46 cases), extensive intraalveolar hemorrhage (10 [22%] of 46 cases), viral pneumonitis (10 [22%] of 46 cases), myocarditis (6 [30%] of 20 cases), and meningoencephalitis (1 [5%] of 18 cases).

IHC assay results. IHC assay results were positive for 27 patients (57%), and of these, 26 (96%) had influenza type A and 1 (4%) had influenza type B. The amount of viral antigens present was considered to be significant for 15 patients (56%)

Table 2. Histopathologic features observed in patients with influenza virus infection.

Histopathologic feature, by site	No. of patients evaluated	No. (%) of patients with feature
Trachea and bronchi	40	…
Submucosal congestion	…	36 (90)
Submucosal mononuclear inflammation	…	29 (73)
Necrosis of bronchial epithelium	…	20 (50)
Submocosal hemorrhage	…	20 (50)
Presence of eosinophils	…	16 (40)
Lung	46	
Mononuclear interstitial inflammation	…	31 (67)
Hyaline membranes	…	31 (67)
Intraalveolar hemorrhage	…	25 (54)
Neutrophilic bronchopneumonia	…	21 (47)
Lymph nodes	36	
Hemophagocytosis	…	18 (53)
Heart	20	
Myocyte necrosis and inflammation	…	6 (30)
CNS	18	
Brain abscesses	…	1 (6)

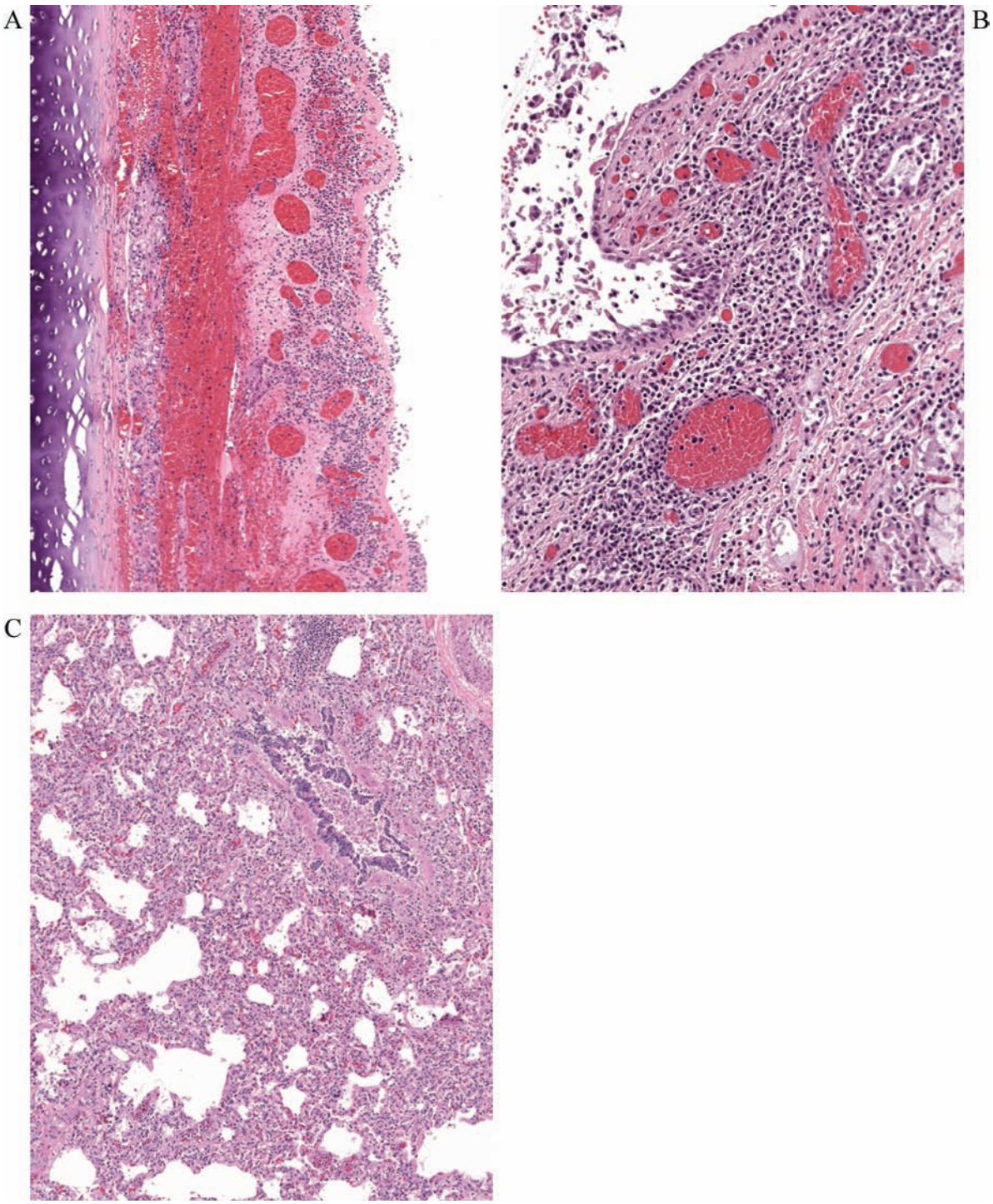

Figure 2. Histopathologic characteristics of influenza A virus infection. *A,* The trachea shows prominent submucosal hemorrhage and congestion (hematoxylin-eosin stain; original magnification, ×40). *B,* The bronchi demonstrate abundant mononuclear inflammatory infiltrate and desquamated necrotic bronchial epithelium (hematoxylin-eosin stain; original magnification, ×20). *C,* The lung shows marked mononuclear interstitial inflammation (hematoxylin-eosin stain; original magnification ×63).

and rare for 12 (44%). Nuclear protein influenza viral antigens were most prominent in the nuclei of infected cells. IHC staining was observed in the bronchoepithelial cells (figure 3*A*) of 25 patients, including 8 patients with staining of mucous glands of trachea, bronchi, and major bronchioli (figure 3*B*). In 6 patients, including 2 without staining of bronchoepithelial cells, viral antigen staining was observed in single cells in the alveoli. In some patients, these cells could be identified as sloughed bronchoepithelial cells by well-defined cilia; however, in other patients, it was difficult to define the cell type because of poor specimen preservation (figure 3*C*).

On average, 5 blocks per patient were studied using IHC assays to test for influenza viruses; among patients with positive IHC assay results, an average of 3 blocks per patient showed influenza viral antigens. Records showed that patients with positive IHC assay results had been ill for a median of 3 days (mean, 3 days; range, 1–15 days; data available for 26 patients), and records show that patients with negative IHC assay results had been ill for a median of 5 days (mean, 5 days;, range, 1–15 days; data available for 20 patients).

Evidence of pneumonia (figure 4*A*) was observed in 21 (47%) of the 46 lung tissue samples available for study. Steiner staining was done on sections from all 21 specimens with evidence of pneumonia; it showed cocci in 4 specimens and bacilli in 1 and was not contributory in 16 specimens. Because of an insufficient number of tissue sections, IHC assays for secondary bacterial and viral pneumonia were performed on only 19 samples. Table 3 presents a correlation of results obtained from histopathologic testing, culture, Gram stain, and IHC assay that defined the significance of these microorganisms as etiologic agents of pneumonia. Nine patients with secondary pneumonia had a clearly established pathogen for the secondary infection, including 3 patients with *S. aureus* infection (figure 4*B*; 2 had methicillin-susceptible *S. aureus*–positive cultures, and in the other patient, the *S. aureus* was not further characterized), 3 with group A streptococci infection (figure 4*C*), 1 with *S. pneumoniae* infection (figure 4*D*), 1 with infection due to *Aspergillus* species, and 1 with *B. pertussis* infection. The patient with infection due to *Aspergillus* species had multiple abscesses in the lung and brain associated with fungal elements (observed with hematoxylin-eosin, Grocott-Gomori methenamine–silver nitrate, and IHC staining). This patient's lung cultures had also grown *S. aureus*; however, IHC assay did not demonstrate the cocci in areas of pneumonia, suggesting that *S. aureus* was colonizing the airways. Similarly, *S. aureus* was isolated from a patient with culture and IHC evidence of group A streptococci, but IHC assay failed to demonstrate *S. aureus* in areas of pneumonia.

One 5-week-old boy with a positive rapid influenza test result showed PCR evidence of *B. pertussis*. The patient had prominent intraalveolar hemorrhage with mild inflammatory infiltrate in the alveoli, and IHC assay confirmed abundant *B. pertussis* antigens in the lung and bronchi. In this context, we tested available samples from other patients who were <2 years old for *B. pertussis*, and all 19 were negative. Results of IHC assays for adenovirus, respiratory syncytial virus, *H. influenzae, N. meningitidis,* and *K. pneumoniae* were negative. Other organisms cultured from specimens from patients with pneumonia included *Enterococcus* species (in 3 patients), *Pseudomonas aureuginosa* (2 patients), *Escherichia coli* (1 patient), *Streptococcus viridans* (1 patient), and *Candida* species (1 patient).

DISCUSSION

This study presents histopathologic and IHC features of 47 samples from pediatric patients with fatal influenza virus infection that occurred during the fall and winter of 2003–2004, and it is the first pathologic study of deaths due to influenza in children during an interpandemic season. In our series, diagnosis of influenza virus infection was established by only 1 test in 40% of patients, and IHC assay was the only diagnostic test in 11% of patients. Laboratory tests are important for the diagnosis of influenza, because organism-specific diagnosis of viral pneumonia cannot be made on the basis of clinical or radiographic features [17, 18]. However, accuracy of rapid influenza diagnostic tests, direct and indirect influenza virus detection (by EIA or direct fluorescent antibody assays), and viral culture may not be sufficiently sensitive by themselves, and use of several methods is frequently necessary to establish a definitive diagnosis of influenza virus infection. Thus, a combination of clinical judgment, local surveillance data, and different laboratory tests are necessary for reliable diagnosis of influenza [18, 19]. This study reaffirms the usefulness of IHC assays as a component in the armamentarium for diagnosis of influenza virus infection in patients who die with respiratory symptoms during the influenza season, and it underscores the importance of performing autopsies in instances where influenza virus infection is suspected. The performance of the IHC assay, compared with the performance of other tests, could not be assessed in this study, because not all tests were performed for all patients.

Our IHC assays detected influenza viral antigens in 27 (57%) of 47 patients who died of influenza. As is consistent with the findings in our previous study [12], the antigens in this study were generally found in few bronchoepithelial cells of bronchi and larger bronchioli. Compared with our initial study, this study of deceased patients from the 2003–2004 season included a large proportion of tissue specimens obtained from the trachea, bronchi, and lung with larger bronchioli. This was because we encouraged sampling of tissue with bronchoepithelial cells to increase the possibility of a positive result by IHC assay. We detected influenza viral antigens in major airways, despite the frequent presence of necrosis and sloughing of epithelial cells in these tissues. To increase the sensitivity of the IHC assay, we

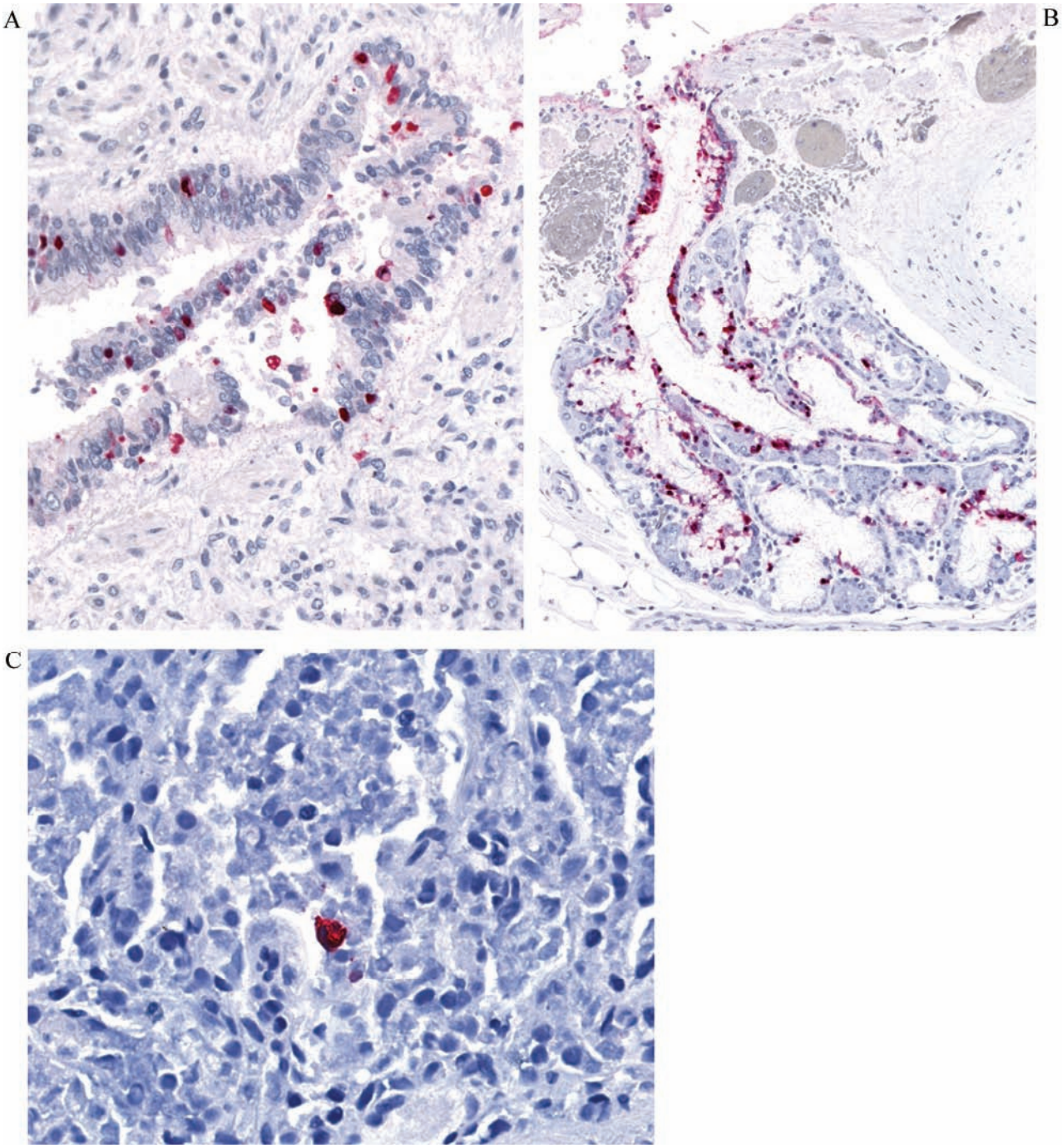

Figure 3. Influenza A virus antigens are present primarily in *(A)* the nuclei of bronchoepithelial cells of bronchioli and in *(B)* the mucosal glands and duct of trachea. Influenza A virus antigens were occasionally observed in single cells in the lung parenchyma *(C)*, probably corresponding to desquamated bronchoepithelial cells that had been aspirated (immunohistochemical assay using a monoclonal anti-influenza A virus nucleoprotein antibody detected with naphthol fast red; original magnifications, ×10 *[A]*, ×20 *[B]*, and ×10 *[C]*).

recommend studying at least 5 blocks prepared from bronchi and centrally located lung samples containing larger bronchioli, particularly those areas that have submucosal inflammation. An average of 3 of 5 blocks from patients with positive IHC assay results had detectable antigen staining.

In our study, positive IHC assay results were most frequently found in patients who died within 3 days of illness onset. This is consistent with findings from studies of experimental human infections in which the proportion of positive viral cultures from nasopharyngeal lavage fluid specimens was highest during

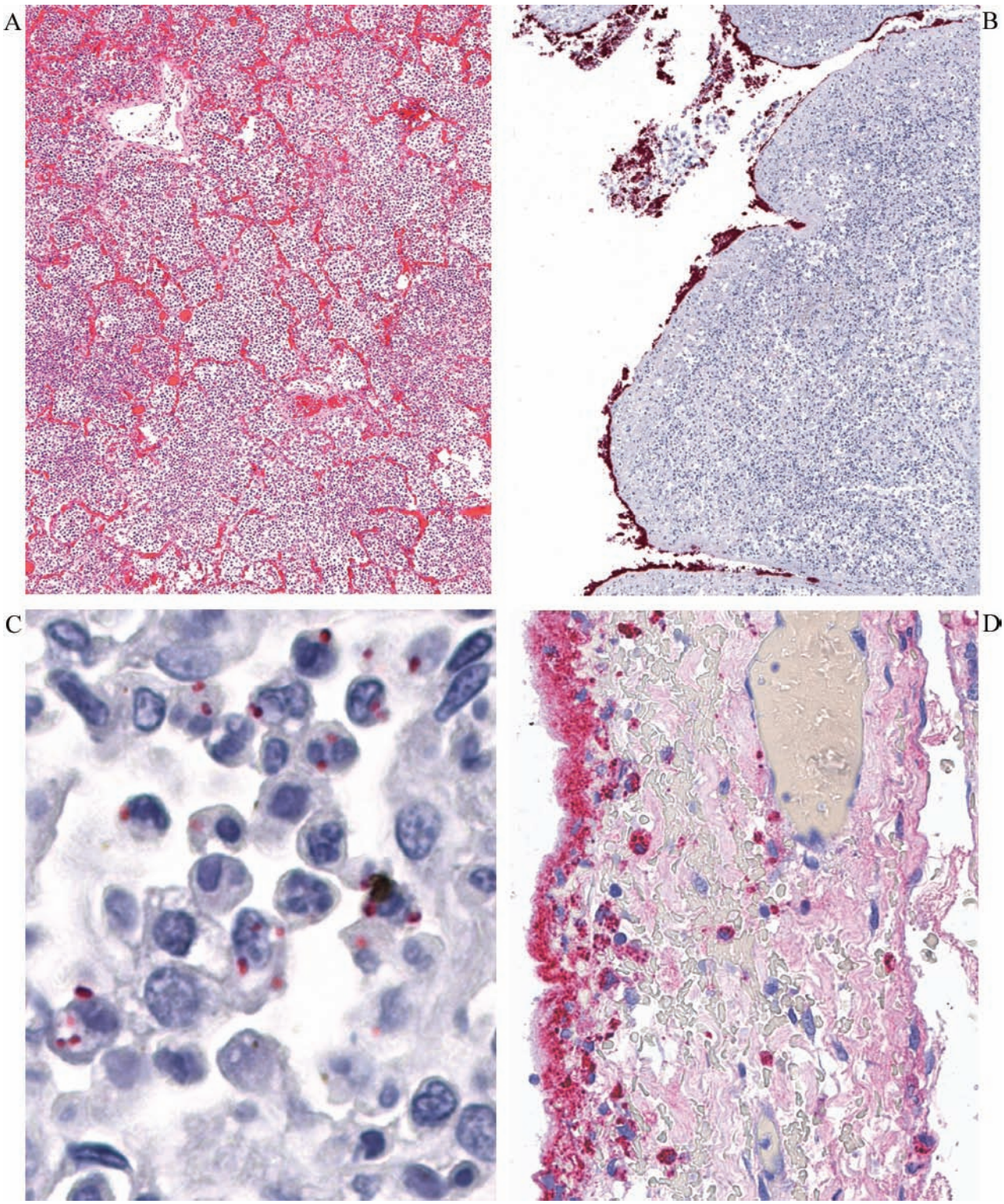

Figure 4. *A,* Histopathologic evidence of secondary pneumonia demonstrated by intense intraalveolar neutrophilic infiltrate (hematoxylin-eosin stain; original magnification, ×10). *B, Staphylococcus aureus* is observed in the surface of bronchioli that show intense inflammation and necrotic bronchial epithelium (immunohistochemical assays using a polyclonal antibodies against *S. aureus;* original magnification, ×10). *C,* Group A streptococci are noted inside inflammatory cells in area of pneumonia (immunohistochemical assays using a polyclonal antibodies against group A streptococci; original magnification, ×63). *D,* Abundant *Streptococcus pneumoniae* antigens and intact bacteria are present in the pleural surface of a patient with secondary pneumonia (immunohistochemical assays using a polyclonal antibodies against *S. pneumoniae;* original magnification, ×63).

Table 3. Microbiological significance of laboratory identification of organisms: correlation of histopathologic feature, culture, Gram stain, and immunohistochemical (IHC) assay results.

Organism	Positive culture results	Gram-positive cocci	Positive IHC assay results
Associated with pneumonia			
Staphylococcus aureus	3[a]	2	3
Group A streptococci	3	1	3
Streptococcus pneumoniae	ND	1	1
Aspergillus species	1	0	1
Bordetella pertussis	ND	0	1
Not associated with pneumonia (colonization)[b]			
S. aureus	2	0	2

NOTE. Data are no. of patients. ND, not done.

[a] Two patients had methicillin-susceptible *S. aureus* infection; the other patient's infection was not further characterized.

[b] These 2 patients include patients described above: 1 had culture results positive for group A streptococci and *S. aureus;* however, by IHC assay, only group A streptococci were observed in the area associated with pneumonia, and *S. aureus* strains were in the airways not associated with bronchopneumonia. The second patient had culture results positive for *Aspergillus* species and *S. aureus;* however, by IHC assay, only *Aspergillus* species were present in the area associated with pneumonia, and *S. aureus* strains were in the airways not associated with bronchopneumonia. Thus, *S. aureus* culture results in these samples were considered to be evidence of colonization.

the first 36 h after the onset of symptoms, but only 13% of nasopharyngeal lavage fluid specimens had positive culture results on day 6 after infection, and all cultures were negative on day 8 [20]. Our findings regarding influenza are similar to those for other human viral diseases in which antigens are usually found at early stages of the infection and disappear as the infections are cleared [21].

For most of our patients, histopathologic study of tissues showed a cause of death, including extensive secondary pneumonia, extensive intraalveolar hemorrhage, diffuse alveolar damage, myocarditis, and meningoencephalitis. However, in 11 (23%) of 47 patients, a clear cause of death could not be determined. The most prominent pathologic condition associated with the presence of influenza virus antigens was inflammation around the trachea, bronchi, and bronchioli, but the amount of virus present seemed too sparse to be the cause of death. Infections of a viral origin are known to exacerbate asthma [22]. Even though only 2 patients had a clinical history of asthma in our series, the presence of eosinophils in 16 (40%) of 40 patients may be considered to be circumstantial evidence of asthma or IgE and cytokine-mediated bronchospasm, conditions that are difficult to diagnose in pathologic postmortem material [23]. In our series, another cytokine-mediated process, hemophagocytosis, was present in 18 (50%) of 36 patients. Several human studies of naturally acquired or experimentally induced influenza demonstrated that respiratory and constitutional symptoms correlate with the presence of multiple cytokines—including IL-4, IL-6 and TNF-α—in plasma or nasopharyngeal fluid, but viremia has not been detected through the disease process [20, 24, 25]. The role these cytokines may play in the development of fatal cases of infection needs further investigation.

During the 1957–1958 H2N2 pandemic, secondary bacterial pneumonia was considered to be the most frequent complication of influenza, and it occurred in ~75% of fatal influenza-related cases [7]. In our study, secondary pneumonia was present in 47% of cases that had a fatal outcome. The lower frequency of secondary pneumonia observed in the 2003–2004 season may reflect the lower distribution of pneumonic complications during interpandemic influenza seasons, changes in access to medical care, better techniques for diagnosis of different viral and bacterial respiratory infections, the availability of pediatric pneumococcal vaccine, and/or the availability of broader antimicrobial treatment.

In our study, bacteria cultured from tissue specimens from patients with influenza included *S. aureus, S. pneumoniae,* group A streptococci, *Enterococcus* species, *H. influenzae,* and *N. meningitides* [4]. We had difficulty correlating culture results with clinicopathologic features of pneumonia, because samples for culture were not systematically obtained, culture results frequently included postmortem samples with growth of mixed bacteria, and there were inherent problems with culture techniques (i.e., inhibition of bacterial growth following antibiotic treatment). IHC assays performed using formalin-fixed, paraffin-embedded tissues offered the advantage of colocalization of specific respiratory pathogens in areas with pathologic conditions. With IHC assays, bacteria were observed inside inflammatory cells in areas with evidence of pneumonia or colonizing the airways. In this series, we identified a respiratory pathogen in ~47% of patients with histopathologic evi-

dence of pneumonia. Most of the bacteria identified were gram-positive respiratory pathogens.

During the 2003–2004 influenza season, 153 influenza-related deaths in children were reported; however, we only received tissues specimens of 47 patients, which may have been subject to selection bias [2]. Our study may have limitations in the diagnosis of influenza, because a variety of influenza tests were used, and 40% of cases were diagnosed on the basis of only 1 positive test result, all of which may have been false-positive or false-negative. Tissue sampling at autopsy was not systematically performed by the different pathologists, which limited possibilities of comprehensive diagnosis, particularly in cases with multifocal pneumonia. In addition, our bacterial IHC testing was targeted to only several specific bacteria, and those not tested for would naturally be missed.

In summary, inflammation and hemorrhage around major airways are frequent pathologic features of fatal influenza virus infections in children. IHC assays were useful for diagnosis of fatal influenza during the 2003–2004 influenza season. To maximize the possibilities of a positive result by IHC assay, we recommend studying at least 5 blocks prepared from bronchi and centrally located lung containing larger bronchioli. Although there are current limitations for IHC detection of bacteria, IHC assays were useful to establish bacterial etiologies of secondary infections. This study underscores the importance of performing autopsies to identify the causes of death in patients with influenza.

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Potential conflicts of interest. All authors: no conflicts.

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