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CASE REPORT

PATHOLOGY/BIOLOGY

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Influenza A/H1N1 (2009) Infection as a Cause of Unexpected Out-of-Hospital Death in the Young*

ABSTRACT: In March 2009, a new strain of influenza A/H1N1 virus was identified in Mexico, responsible for a pandemic. Worldwide, more than 13,500 patients died, most often from acute respiratory distress syndrome. Because sudden death cases were rare, involving mostly young apparently healthy persons, influenza A/H1N1 (2009)-related deaths may be misdiagnosed, which can raise medico-legal issues. Case history: we report on an unexpected out-of-hospital death involving a young male with no past medical history and no vaccination. Fever was his only symptom. Laboratory tests: histology showed patchy necrotic foci with mononuclear inflammation in the lungs. The heart was histologically normal, but virological analyses using molecular biology on frozen myocardial samples showed high virus load. In conclusion, this case report shows that influenza A/H1N1 (2009) virus can be a cause of sudden cardiac death in the young and demonstrates the importance of quantitative virological analyses for the diagnosis of myocarditis.

KEYWORDS: forensic science, forensic pathology, autopsy, influenza A, pandemic, sudden unexplained death, virology, myocarditis, postmortem

In March 2009, a new strain of influenza A/H1N1 virus was identified in Mexico, combining the genetic characteristics of human, avian, and swine influenza A viruses. Between June 11, 2009, and August 10, 2010, an influenza pandemic was declared by the World Health Organization. Worldwide, more than 13,500 patients died of influenza A/H1N1 (2009) infection (1). The death prevalence was similar in seasonal influenza as well as influenza A/H1N1 (2009) infections (0.3‰), but the decedents' characteristics were different (2,3). Influenza A/H1N1 (2009) involved mostly young apparently healthy patients. Most of the deaths occurred in patients hospitalized with respiratory symptoms, fever, and digestive symptoms, including diarrhea and vomiting (3–11). Antemortem diagnosis was assessed by RT-PCR testing of nasopharyngeal swab specimens collected on admission to hospital according to published guidelines from U.S. Centers for Disease Control and Prevention (CDC protocol of real-time RT-PCR for influenza A H1N1 [2009]) (2,3,5,7–10,12–14). Ordinary seasonal influenza rarely leads to death, and thus, few cases exist to allow comparison of histology from cases of ordinary seasonal influenza with cases of pandemic influenza (7). Only a few studies with a limited number of cases have been published describing the histopathological findings of seasonal influenza in humans (7). Sudden death cases, especially out-of-hospital deaths, were rare. Because symptoms are unspecific, and sudden deaths involve mostly young apparently healthy persons, influenza A/H1N1 (2009)-related deaths may be misdiagnosed, which can raise medico-legal issues. We report on an unexpected out-of-hospital death involving a young healthy male. Histopathological lesions and virological analyses are examined.

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Case Report

By the end of December 2009, a 19-year-old man was found dead in his bed, in the morning by his mother. Fever (38.4°C) was his only symptom when he went to bed in the evening. He had no past medical history and was not a drug abuser. His height and body weight were 1.76 m and 67 kg (BMI = 21.7), respectively. Body examination at the scene was normal.

He had not received vaccination against influenza A/H1N1 (2009).

Autopsy Findings

Lungs were found increased in weight (right, 760 g; left, 520 g). They were dark red, suggesting congestion/focal hemorrhage. The heart was normal in weight (340 g). No lesions were found. Other organs were otherwise normal. Both lungs, the whole brain and heart, and samples of tonsil, larynx, trachea, bronchi, spleen, liver, kidney, thyroid, and adrenal glands were collected for microscopic examination.

Histology

Histological examination of the larynx, trachea, bronchi, and bronchioles showed mild acute inflammation. Histological

examination of the lungs (14 samples) showed patchy foci of necrosis and inflammation. These foci were grossly rounded and well circumscribed. Inflammatory infiltrates consisted of mononuclear cells, lymphocytes, and macrophages associated with rare multinucleated cells (two to three nuclei) and neutrophils. There were no diffuse alveolar damage lesions. Elsewhere, alveolar spaces were preserved, but focally hemorrhagic, with some macrophages and congestive septa. There was no evidence of diffuse alveolar damage (Fig. 1). Other organs, including the heart (15 samples), were otherwise normal.

Microbiology

Nasal swabbing was performed for viral analyses according to published guidelines from U.S. Centers for Disease Control and Prevention (CDC protocol of real-time RT-PCR for influenza A H1N1 [2009]). Fresh and frozen lung samples were collected for bacterial and viral analyses, respectively. Frozen heart samples were collected for viral analyses according to international guidelines (15).

Bacteriology

Polymorphic bacterial flora without predominance was found in lung cultures, of no pathological significance.

Virology

Detection of Human Respiratory Viruses—The NucliSENS-easyMAG instrument (BioMérieux, Lyon, France) was used for total nucleic acid extraction, according to the manufacturer's protocol.

Two RT-PCR DNA microarray detection systems (16) were used in combination for the detection of human respiratory viruses in the frozen heart and lung samples. Clart Pneumo Vir and Clart FluA Vir kits (Genomica, Madrid, Spain) allowing simultaneous detection of 21 different types and subtypes of human respiratory viruses (influenza A virus [seasonal A/H1N1 and A/H3N2, and new influenza A/H1N1 (2009) virus strains], influenza B virus, influenza C virus, parainfluenza virus 1, 2, 3, 4, 4A and 4B, respiratory syncytial virus A and B, rhinovirus, adenovirus, enterovirus type B, bocavirus, coronavirus E-229, metapneumovirus A and B) were used according the manufacturer's protocol.

Influenza A/H1N1 (2009) virus was the only virus detected in all samples (nasal swabs, lungs, and heart).

Identification and Quantification of the Influenza A/H1N1 (2009) Virus—Real-time RT-PCR assays (SuperScript III Platinum one-step quantitative RT-PCR system; Invitrogen, Carlsbad, CA) were performed for the identification and quantification of the influenza A/H1N1 virus (17). The virus was identified in all samples. The viral load was 2.12×10^6 copies/mL in the nasal swabs, 7.56×10^5 copies/μg extracted DNA in the lungs, and 1.41×10^4 copies/μg extracted DNA and 8.82×10^3 copies/μg extracted DNA in the left and right ventricles, respectively.

Sequencing Assay of the Hemagglutinin and Neuraminidase Influenza Genes for Identification of Specific Mutations of the Influenza A/H1N1 Virus—There was no polymorphism involving the amino acid 275Y of the neuraminidase, known to cause oseltamivir resistance. Also, there was no polymorphism involving the amino acid 222D of the hemagglutinin glycoprotein, known to cause a highly pulmonary virulence of the influenza strain (18).

Biochemistry and Toxicology

Peripheral and cardiac blood, as well as vitreous humor, was collected for biochemical and toxicological analyses.

No illicit nor prescribed drugs were found.

Biochemical study of electrolytes, glucose, and organic acids (gas chromatography and mass spectrometry) in vitreous humor did not reveal electrolytic nor metabolic disturbances.

Discussion

We have reported on an unexpected sudden out-of-hospital death caused by influenza A/H1N1 (2009) infection involving a young immunocompetent and previously healthy male. Influenza A viruses are known to cause myocarditis (19,20), but published cases of influenza A/H1N1 (2009)-related myocarditis are rare and not thoroughly documented (2,6). In our case, virological investigations were performed, including molecular analyses of common pulmonary and cardiotropic viruses in frozen cardiac samples, as well as genetic analysis of the influenza A/H1N1 (2009) virus for both hemagglutinin and neuraminidase genes.

Influenza A viruses are human respiratory pathogens that cause seasonal endemic outbreaks and/or periodic unpredictable pandemics. Pandemics are typically associated with influenza A virus strains harboring a novel form of the hemagglutinin molecule (19). In March 2009, infections caused by a new swine-origin influenza A/H1N1 virus were diagnosed in Mexico and the United States (4). In June 2009, the World Health Organization declared influenza pandemic. Autopsy and epidemiological studies showed some differences between seasonal and pandemic influenza infections (19). Age at the death was younger than in the seasonal form, as in

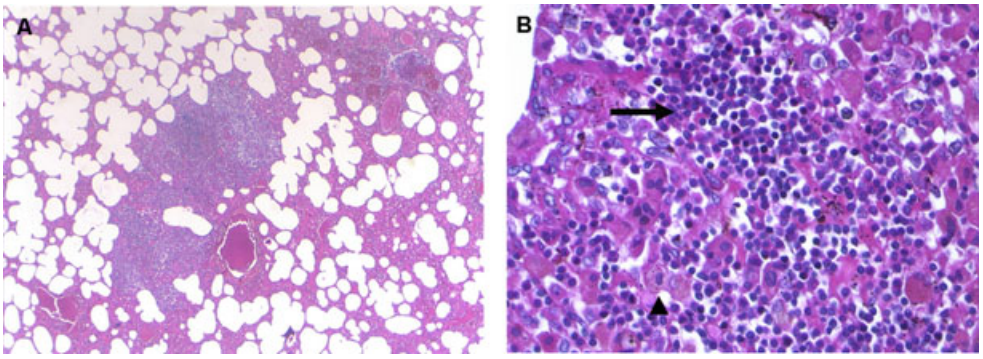

FIG. 1—*(A, B) Lungs. Hematoxylin, eosin-stained sections show scattered foci of necrosis and inflammation (×40) (A). The inflammatory cells consisted mainly of mononuclear cells: lymphocytes (arrow) and macrophages (arrow head) (×400) (B).*

our case (2–13). Immunologic protection induced by prior exposure to previously circulating A/H1N1 viruses (5) is likely to explain this age difference. Up to 91% of the patients had preexisting medical conditions, including cardiac or respiratory diseases, immunosuppression, pregnancy, and obesity. The latter was an unexpected finding as compared to prior pandemics (5).

During the influenza pandemic, most of the deaths occurred in hospitalized patients with previous flu-like symptoms. Acute respiratory distress syndrome was the cause of most of these deaths (3,5,7–13). Although thousands of patients died from the infection worldwide, autopsies were rare (Table 1). Diagnosis was based on molecular virological analyses performed in nasal swabs according to published guidelines from U.S. Centers for Disease Control and Prevention (CDC protocol of real-time RT-PCR for influenza A H1N1 [2009]), but pulmonary and cardiac tissue virological analyses were not performed systematically. Consequently, the mechanisms by which the virus caused lethal lesions were not thoroughly investigated.

Only one unexpected out-of-hospital death has been reported (2). The young female victim had previous flu-like symptoms and diarrhea for a week. She was found dead in her living room with the television on, which suggested a sudden arrhythmic death. Nonetheless, pneumonia was assessed as the cause of the death, because of histological findings. The heart was both macroscopically and histologically normal, but only oral cavity and upper respiratory tract swabs for the influenza virus were performed for virology. The possible presence of the virus in the heart and its proarrhythmogenic role was not investigated. Yet, some studies have shown that cardiotropic viruses may cause acute/fulminant myocarditis without inflammatory cells (21–24). Influenza A/H1N1 (2009)-related myocarditis was assessed as the cause of two reported unexpected out-of-hospital deaths (2,6). In one case, there was no evidence of pneumonia, whereas in the other, the child patient had both pneumonia and myocarditis. Histological myocardial inflammation was present in both cases. In both cases, influenza A/H1N1 (2009) virus was found by PCR in the heart. However, in one case, no other cardiotropic viruses were sought in the heart to rule out the involvement of such viruses. In the other, cardiotropic viruses were sought in paraffin-embedded cardiac tissue. It has been shown that virological analyses should be performed on frozen tissue, because of false-negative results when paraffin-embedded tissues are used (15). Furthermore, in both cases, influenza A/H1N1 (2009) virus load was not quantified.

In our case, death was unexpected. Fever was the only symptom. In particular, the person did not complain of dyspnea nor diarrhea, and the death occurred during sleep, which indicates an arrhythmogenic mechanism of death. In this context, and because the death involved a young person with no past medical history nor drug abuse, the death was considered suspicious. Influenza A/H1N1 (2009) virus was the only virus found in the heart despite thorough analyses on frozen cardiac samples for all cardiotropic viruses.

The first descriptions of microscopic findings in H1N1 infection were from the 1918 influenza pandemic (19). Diffuse alveolar damage was the most common pathologic finding (19). Alveolar hemorrhages in patients with comorbidities and/or acute bacterial bronchopneumonia were also frequent findings associated with the influenza A/H1N1 (2009) virus (2–5,7–9,11). Acute necrotizing tracheobronchitis and/or bronchiolitis, sometimes associated with hemorrhage is a frequent finding (4,5,7–9). These patterns are not specific of influenza A/H1N1 (2009) infection, being also found in the seasonal influenza-related deaths (19). In our case, the microscopic lung findings were unusual. No diffuse alveolar damage, but rounded patchy foci of necrosis associated with inflammatory cells were found. These cells were mostly lymphocytes and mononuclear macrophages, associated with some multinucleated (two to three nuclei) macrophages. Neutrophils were very rare. In the trachea and bronchi, only mild and focal inflammation was present. Not surprisingly, the person had no respiratory symptoms. Influenza A/H1N1 (2009) virus was the only cause of pulmonary lesions in this person, because thorough microbiological investigations were performed on frozen lung tissue.

Influenza A viruses are known to cause myocarditis (19,20), but published cases of influenza A/H1N1 (2009)-related myocarditis are rare and not thoroughly documented (2,6). In the published cases, acute myocarditis was characterized by necrosis and inflammation, associated with the detection of influenza A/H1N1 (2009) by PCR (2,6). The possible presence of other cardiotropic viruses in the heart was not investigated. Furthermore, some studies have shown that cardiotropic viruses may cause acute/fulminant myocarditis without inflammatory cells (21–24). In our case, we did not find myocardial inflammation despite many samples in both ventricles (n = 15). According to Dallas criteria used for endomyocardial biopsy examination, myocarditis has been defined as inflammation associated with necrosis (25). It is now recognized that the Dallas criteria are not sensitive for the diagnosis of myocarditis, because they do not take into account the presence of viral genome in the heart (19,26). Investigators have shown that virus may be present in the myocardium without Dallas myocarditis criteria. Martin et al. (21) demonstrated in 34 children with clinical presentations compatible with myocarditis that 26 heart biopsy samples were positive for viral pathogens, and 13 of the 26 positive samples had no evidence of myocarditis by histopathological examination. A meta-analysis of PCR studies in patients who had heart biopsies with presumed myocarditis or cardiomyopathy demonstrated an odds ratio of 3.8 for viral presence in both categories compared with control patients (22). In a selected sample of 45 patients with left ventricular dysfunction and suspected myocarditis and 26 controls, nonreplicative enterovirus was demonstrated in 18 of 45 patients (40%) compared with none of the controls. Of the 18 patients with nonreplicative virus, 10 (56%) were found to have active viral replication as well (23). Therefore, virus can exist in the myocardium in the absence of myocardial inflammation adequate to meet Dallas criteria and may adversely affect outcome (19). In these cases, as in ours, death may have occurred before inflammation developed. The fulminant course in our case suggests high virus virulence and/or non-cell-mediated inflammation. As far as virus virulence is concerned, our genetic investigations did not reveal gene mutations involving hemaglutinin and neuraminidase. However, the patient had not received vaccination.

As far as the mechanism of inflammation is concerned, it has been suggested that the injury to the respiratory epithelium in this disease was attributed to a great release of cytokines, which is sometimes called ''cytokine storm'' (10). Our cardiac findings support this hypothesis. In fulminant forms of the disease, this cytokine storm might explain cardiotoxicity and proarrhythmogenic effects of the virus, before or despite inflammatory cell-mediated involvement.

As far as the pathogenicity of the virus is concerned, the question was raised of whether the presence of the virus is sufficient in assessing its role in the death. In our case, influenza A/H1N1 (2009) virus was detected in pharyngeal swabs, frozen lungs, and heart samples. Viral load quantification showed high levels of viral genome copies per microgram of extracted DNA in the different collected samples. In only one other study, viral load was quantified in paraffin-embedded lung, bronchus, and tracheal tissues (12). The number of viral influenza A/H1N1 (2009) copies was

TABLE 1—*Patients characteristics, symptoms, autopsy findings, histological findings, virological methods, and bacteriological findings in the published cases of influenza A/H1N1 (2009)-related deaths.*

References	Number of Cases and Gender	Ages	Past Medical History	Symptoms	Autopsy Findings	Histology	Virological Methods	Bacteriology
Edler et al. (2)	2F	13–31 years	No	Collapse (n = 1) No (n = 1)	Congested lungs	Acute pneumonia (n = 1), acute myocarditis (n = 1), and alveolar hemorrhage (n = 1)	RT-PCR in nasal swab and lungs, heart, and brain tissues	NS
Harms et al. (3)	8M	23–57 years	Respiratory distress (n = 4)	Fever (n = 7), cough (n = 4), chills (n = 3), dyspnea (n = 3), and diarrhea (n = 2)	Congested lungs and thromboemboli (n = 5)	DAD (n = 8), thrombosis (n = 7), and alveolar hemorrhage (n = 1)	RT-PCR in nasal swab and frozen lung tissue	*Acinetobacter baumannii* (n = 1)
Soto-Abraham et al. (4)	5*	22–83 years	NS	NS	Congested lungs	Tracheobronchitis, DAD and thrombosis (n = 4), alveolar hemorrhage (n = 1), and acute pneumonia (n = 3)	rRT-PCR	NS
Gill et al. (5)	17F/17M	2 months–60 years	Cardiopulmonary comorbidities (n = 29)	Fever (n = 30), cough (n = 31), respiratory distress (n = 24), and gastrointestinal symptoms (n = 11)	NS	Tracheitis and necrotizing bronchiolitis, DAD, alveolar hemorrhage, and thrombosis (n = 9), and acute pneumonia (n = 19)	RT-PCR in FFPE lungs and trachea (n = 23), and in nasal swab (n = 28)	*Streptococcus pneumoniae* (n = 6), *Staphylococcus pyogenes* (n = 3), MRSA (n = 1)
Gdynia et al. (6)	1F	18 years	No	Flu-like and diarrhea	Slight pallor of heart and kidney	Acute myocarditis	rRT-PCR in blood, heart, lung, and small-bowel tissues, and nested PCR in FFPE heart tissue	NS
Rosen et al. (7)	8M	6 months–54 years	Sleep apnea (n = 1)	Flu-like (n = 8) and fever (n = 5)	Laryngeal and tracheobronchial edema, and congested lungs	Necrotizing bronchiolitis, DAD, and alveolar hemorrhage	Rapid influenza diagnostic test, and RT-PCR in FFPE lungs and upper airway tissues	*Pseudomonas aeruginosa* and Streptococcus B (n = 1)
Shieh et al. (8)	49F/51M	2 months–84 years	Asthma (n = 22) and cardiovascular disease (n = 25)	Flu-like (n = 100)	NS	Tracheitis (n = 66), DAD (n = 59), alveolar hemorrhage (n = 58), and acute pneumonia, (n = 29) and thrombosis (n = 17)	rRT-PCR in fresh, frozen or FFPE tissue samples (lung, heart, kidney, liver, brain, spleen, gastrointestinal tract, muscle, and pancreas)	*S. pneumoniae* (n = 10), mitis (n = 2), and agalactiae (n = 1), *S. pyogenes* (n = 5) and MRSA (n = 4)
Mukhopadhyay et al. (9)	1F/1M	36–46 years	HTA, smoke, and alcoholism	Flu-like (n = 1) and coma (n = 1)	Congested lungs	DAD, alveolar hemorrhage, and focal acute pneumonia	rRT-PCR in lungs, nasal swab, heart, and brain	*S. pneumoniae* (n = 1)
Calore et al. (10)	1F/5M	23–48 years	HTA (n = 1)	Flu-like (n = 5)	Congested lungs	DAD (n = 4), thrombosis (n = 2), organized bronchopneumonia (n = 2), and myocarditis	RT-PCR in nasal swab	NS
Springer et al. (11)	2F/4M	8–53 years	Asthma and bronchodysplasia (n = 1)	Flu-like (n = 5)	Congested lungs	Necrotizing bronchiolitis, DAD, and alveolar hemorrhage, and microthrombi (n = 4)	NS	*Citrobacter koseri* and *Klebsiella pneumoniae* (n = 1)

Continued.

TABLE 1—*Continued.*

References	Number of Cases and Gender	Ages	Past Medical History	Symptoms	Autopsy Findings	Histology	Virological Methods	Bacteriology
Nakajima et al. (12)	1M	33 years	Asthma and cardiovascular	ARDS	Congested lungs	DAD	qrRT-PCR in FFPE lung and tracheal tissue	NS
Mauad et al. (13)	9F/12M	1–68 years	Cardiovascular (n = 7), immunosuppression (n = 2), and respiratory distress (n = 2)	Respiratory distress (n = 21)	Congested lungs	DAD (n = 20) and thrombosis (n = 4)	rRT-PCR in nasal swab and lung tissues	*Hemophilus influenzae* and *S. pneumoniae* (n = 3)

ARDS, acute respiratory distress syndrome; DAD, diffuse alveolar damage; FFPE, formalin-fixed paraffin-embedded tissue; HTA, hypertension; NS, nonspecified; rRT-PCR, real-time RT-PCR; qrRT-PCR, quantitative real-time RT-PCR.
*Gender not specified in article.

2.37×10^5, but the site where this load was assessed was not specified (12). Furthermore, extraction of good-quality DNA and RNA from formalin-fixed, paraffin-embedded (FFPE) tissues is compromised because of incomplete removal of protein–nucleic acid cross-links (27). Other authors performed real-time RT-PCR on nonrespiratory FFPE tissue and failed to detect influenza A/H1N1 (2009) (8). Impaired nucleic acid can lead to false-negative result. It has been shown that quantification of viral load in different tissues provides information on the virus spreading pattern (28). In one study, a high number of viral copies per cell in the trachea, compared with a small number in the lower lung samples, suggested a gradient of viral replication throughout the respiratory tract (12). In our case, a higher viral load in the upper respiratory airways than in the lung was also found.

Myocarditis has been recognized as one of the main causes of sudden death in the young (29–31). It has long been underestimated because of the lack of autopsies. Forensic pathologists have played an important role in improving the knowledge of the different patterns. In most cases, the heart is grossly normal. Histology requires many samples in both ventricles because of the patchy inflammatory pattern (19,29–31). More recently, molecular biology has shown the importance of virological analyses performed on frozen tissue, including quantification of viral load (15,19,20,26). In our case, determination of viral loads in the absence of any inflammatory infiltrate in the myocardium has shown that influenza A/H1N1 (2009) virus can be a cause of out-of-hospital unexpected death in the young. Because symptoms are sometimes unspecific and mild, influenza A/H1N1 (2009)-related deaths may be misdiagnosed, which can raise medico-legal issues. As far as epidemiological and virological issues are concerned, autopsies and thorough virological investigations are useful in understanding pandemics and the virus virulence.

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