

Human Infection with Highly Pathogenic Avian Influenza A (H5N1) Virus: Review of Clinical Issues

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This article provides an updated review of the clinical issues related to human infection with highly pathogenic avian influenza A (H5N1) virus. The clinical data available to date are presented, as well as recent findings on the pathogenesis of and antiviral treatment and immunotherapy for H5N1 virus infection in humans and animal models.

The first recognized case of human illness from infection with highly pathogenic avian influenza A (H5N1) virus occurred in May 1997 [1, 2], and 17 additional cases were detected in late 1997 in Hong Kong; overall, there were 6 deaths [3]. Two cases were identified in February 2003 among Hong Kong family members who traveled to southern China [4]. From November 2003 through 2 June 2009, there were 433 sporadic cases of H5N1 virus infection reported from 15 countries [5]; in 262 (61%) of those cases, the patient died. These cases were associated with the ongoing H5N1 panzootic among poultry and have raised concerns of a possible H5N1 influenza pandemic, stimulating global preparedness and response activities. Recent reviews have summarized the development of H5N1 vaccines and their potential use [6–8]. This article reviews the available clinical data to date.

PATHOGEN

Highly pathogenic H5N1 virus has evolved, through a complexity of genetic changes, from the 1996 progenitor strain and comprises at least 10 groups, or clades, of antigenically and genetically distinct strains that have infected domestic poultry and wild birds in many countries [9–12]. To date, 4 clades (0, 1, 2, and 7) and 3 subclades (2.1, 2.2, and 2.3) of H5N1 virus

strains have infected people [13]. During the period from late 2003 to mid-2005, most H5N1 virus infections in humans were caused by clade 1 strains in Southeast Asia (i.e., Vietnam, Thailand, and Cambodia). Beginning in 2004–2005, a geographic expansion of clade 2 H5N1 virus strains circulating among poultry and wild birds occurred from Asia to Europe, the Middle East, and Africa [14]. Currently, subclade 2.1 virus strains are circulating among poultry and have caused human infections in Indonesia, whereas subclade 2.2 virus strains have infected birds and humans in Africa, Asia, and Europe [13]. Subclade 2.3 virus strains have been detected in poultry in China and nearby Southeast Asian countries with transmission to humans [10, 13, 15]. It is unknown whether differences exist between H5N1 virus strains (by clade or subclade) in the risk of avian-to-human transmission. The highly pathogenic H5N1 virus strains can be expected to continue evolving.

H5N1 virus is thought to bind primarily to receptors on distal bronchiolar and alveolar cells (type II pneumocytes and macrophages) expressing SA- α -2,3-Gal (sialic acid bound to galactose by α -2,3 linkages) [16, 17]. However, these receptors have also been reported in tracheal tissue, nasal mucosa, pharynx, bronchi, paranasal sinus, neuronal, intestinal, hepatic, splenic, renal, epithelial, and vascular endothelial tissue cells, T cells, and neonatal respiratory tissues [18–20]. Additionally, H5N1 viral replication was shown in ex vivo nasopharyngeal, adenoid, and tonsillar tissue cultures without detectable SA- α -2,3-Gal receptors [21], and H5N1 virus strains were isolated from samples obtained from 4 patients that had the ability to bind to upper respiratory tract SA- α -2,6-Gal receptors [22, 23]. Chandrasekaran et al. [24] demonstrated that a specific structural conformation, not the SA- α -2,6-Gal linkage alone, de-

Received 1 December 2008; accepted 7 March 2009; electronically published 12 June 2009.

This article reflects the viewpoints of the author and not necessarily those of the Centers for Disease Control and Prevention.

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Clinical Infectious Diseases 2009;49:279–90

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1058-4838/2009/4902-0020

DOI: 10.1093/cid/cin35

termines viral binding to upper respiratory tract SA- α -2,6-Gal receptors, illustrating the complexity of receptor specificity.

DIAGNOSIS

Critical issues in diagnosing acute H5N1 virus infection are to determine which patient to test (on the basis of epidemiological and clinical findings), to collect proper clinical specimens, and to use appropriate testing methods. The World Health Organization (WHO) issued definitions for the classification of suspect, probable, and confirmed cases of H5N1 virus infection, for epidemiologic and reporting purposes [25]. Risk factors for H5N1 virus infection are direct physical contact with or close exposure (i.e., <1 meter) to sick or dead poultry in the week before illness onset [26–28] and visiting a live poultry market [28–30]. However, for some cases, exposure to H5N1 virus was not identified [31]. In a small number of cases in clusters, limited, nonsustained human-to-human transmission of H5N1 virus likely occurred [32–34]. Therefore, H5N1 virus infection should be considered in a person with febrile, acute respiratory illness in countries where highly pathogenic H5N1 poultry outbreaks have occurred, for whom there is a recent history of direct or close exposure to sick or dead poultry, who has visited a live poultry market, or who has had close contact (within 1–2 meters) with an individual with H5N1 virus infection. Clinical diagnosis during early H5N1 illness is challenging because of the nonspecific signs and symptoms and rarity of H5N1 disease. No cases of H5N1 virus infection have been identified in travelers to date, and seasonal influenza A virus infection was diagnosed in 25 (42%) of 59 returned US travelers with suspected H5N1 [35]. The Centers for Disease Control and Prevention (CDC) has issued guidelines for testing suspected cases of H5N1 virus infection in the United States [36].

Diagnostic specimens. For patients who do not receive mechanical ventilation, both throat and nasal swab specimens should be collected. Throat swab specimens appear to have a higher yield for the detection of H5N1 virus than do nasal or nasopharyngeal specimens [37], but viral RNA detection or isolation from nasopharyngeal or nasal specimens of critically ill patients was reported [37–39]. Because H5N1 virus binds to and replicates primarily in lower respiratory tract tissue [16, 17], an endotracheal aspirate specimen should be obtained from patients who receive invasive mechanical ventilation. Pleural fluid and bronchoalveolar lavage specimens, if available, can also be tested. Specimens should be collected from multiple respiratory sites on consecutive days from patients with suspected H5N1 virus infection. Blood or stool specimens should not be tested for diagnostic purposes, but detection of H5N1 viral RNA in these specimens suggests a poor prognosis [37].

Diagnostic testing. The primary method to confirm acute H5N1 virus infection is detection of viral RNA in respiratory specimens by use of reverse-transcription polymerase chain re-

action (RT-PCR) [40, 41] (table 1). Real-time and conventional RT-PCR can be performed under biosafety level 2 conditions. A standardized protocol and reagents to detect H5N1 virus [40] and an H5 real-time RT-PCR primer and probe platform (developed by the CDC) were cleared for distribution [42]. Key laboratory issues include proper RNA extraction, use of updated primers and probes (because H5N1 strains are evolving), use of proper controls, and optimization of the RT-PCR assay [43]. Viral isolation should be performed under enhanced biosafety level 3 conditions, which can be performed at WHO H5 Reference Laboratories [41, 44, 45]. Genomic sequencing of viral RNA from clinical specimens, from amplified RNA, or from H5N1 isolates is important to assess genetic reassortment, and to detect mutations that may affect transmissibility or antiviral resistance. The antigenic characterization of viral isolates is critical for monitoring the evolution of H5N1 virus strains and for the development of vaccines. Commercially available rapid diagnostic tests have poor sensitivity and specificity for detecting H5N1 virus infection and are not recommended [40, 46].

Serological testing can diagnose H5N1 virus infection retrospectively and can confirm RT-PCR results. The recommended serological test is the microneutralization assay [40, 41, 47], which requires the use of live H5N1 virus, to detect neutralizing antibodies, and enhanced biosafety level 3 conditions. Limited data suggest that H5N1-neutralizing antibodies are detectable in serum samples 10–16 days after onset of illness [48]. The collection of paired acute (obtained within 1 week after onset of illness) and convalescent (obtained 2–3 weeks later) serum samples are needed. A single positive H5N1 antibody titer in a convalescent serum sample may help establish a retrospective diagnosis of H5N1 virus infection with a clinically compatible illness but cannot determine the timing of infection. The WHO has published criteria for seropositive results [41]. The use of a modified horse red blood cell hemagglutinin-inhibition assay [49] can confirm the results of the microneutralization assay [38]. However, this modified horse red blood cell hemagglutinin-inhibition assay as well as other serological tests need further validation [50, 51] (table 1).

CLINICAL CHARACTERISTICS OF H5N1 VIRUS INFECTION

Incubation period. The incubation period for H5N1 virus infection has been estimated to be up to 7 days, but, more commonly, it is usually 2–5 days after the last known exposure to sick or dead poultry [26, 48, 52, 53]. However, longer periods have been suggested [26, 27], and the incubation period after visiting a live poultry market was estimated to be 8.5 days [54]. In cases for which limited human-to-human transmission likely occurred, the incubation period was estimated to be 3–4 days [32], 4–5 days [34], 8–9 days [32], and 2–10 days [33]. This

Table 1. Methods for detecting human infection with highly pathogenic avian influenza A (H5N1) virus.

Purpose, assay	Comments
Detection of H5N1 viral RNA^a	
Real-time RT-PCR and conventional RT-PCR	Real-time RT-PCR is the recommended diagnostic test for respiratory (throat, nasal, nasopharyngeal, and endotracheal aspirate) specimens from patients with acute H5N1 virus infection; highly sensitive and highly specific; may be performed under biosafety level 2 conditions conditions; real-time RT-PCR can yield quantitative viral shedding data. RT-PCR methods require updated primers and probes; can be performed on other clinical specimens (blood, serum, plasma, pleural fluid, bronchoalveolar lavage fluid, cerebrospinal fluid, stool, bronchoalveolar lavage fluid, and autopsy tissues).
Isolation of H5N1 virus	
Viral culture (using embryonated eggs) or tissue cell culture	Should only be performed in approved biosafety level 3 enhanced laboratory conditions by experienced personnel wearing appropriate personal protective equipment.
Detection of H5N1 viral antigen	
Rapid influenza diagnostic test (immunoassay)	Not recommended; poor sensitivity and poor specificity.
Immunofluorescence	Lower sensitivity and poor specificity.
Immunohistochemical staining	Used for autopsy tissue specimens.
Detection of H5N1 virus–neutralizing antibodies	
Microneutralization assay	Gold standard serological test; ^b uses live H5N1 virus and is highly specific; generally requires paired acute and convalescent serum samples; confirm with modified horse red blood cell hemagglutinin-inhibition assay; or can also use Western blot or single radial hemolysis assay; can establish retrospective diagnosis of H5N1 virus infection; the H5N1 virus strain used in the microneutralization assay should match or be antigenically similar to the strain that the patient was exposed to or infected with, because this assay is highly specific; humoral immune response to H5N1 virus infection is not well defined, and understanding is limited by high mortality and paucity of follow-up serological data from survivors; should only be performed in approved biosafety level 3 enhanced laboratory conditions by experienced personnel wearing appropriate personal protective equipment.

^a Other related assays used by research laboratories include H5 TaqMan real-time reverse-transcription polymerase chain reaction (RT-PCR), and RT-H5 loop-mediated isothermal amplification.

^b Other related assays used by research laboratories include microneutralization assay using reverse genetics-engineered attenuated H5N1 virus, enzyme-linked immunosorbent assay, and pseudotype viral particle assay.

variability in incubation period could reflect the level of exposure, multiple exposures, immunological factors, or other factors.

Features of early illness. Available detailed clinical data are limited. In most cases, during the early stage of H5N1 virus infection, fever or feverishness is present. Other early symptoms include cough, malaise, myalgia, headache, sore throat, abdominal pain, vomiting, and diarrhea [15, 31, 48, 52, 53, 55–58]. Conjunctivitis is very rare [53, 59]. In some cases, the initial signs and symptoms were fever and diarrhea [57, 60], and some individuals with H5N1 virus infection were diagnosed with typhoid fever or dengue [13]. A pediatric patient with H5N1 virus infection presented with fever, diarrhea, and seizures progressing to coma and was diagnosed with encephalitis [58]. It has been reported that pediatric patients with a clinically mild case of H5N1 virus infection have presented with febrile acute upper respiratory tract illness [39, 48, 53, 59, 61]. Whether the severity of illness varies by clade or subclade of H5N1 virus infection, by age, or by immunological, genetic, or other factors is unknown.

Findings and complications at hospital admission. As the

clinical course of H5N1 virus infection progresses in severe cases, fever and nonproductive cough are often followed by dyspnea, shortness of breath, tachypnea, and chest pain, reflecting the progression of lower respiratory tract disease [15, 31, 38, 53]. The majority of patients (median age, 20 years [range, <1–81 years]) had no underlying medical conditions [62]. Rapid disease progression is observed in most cases. Generally, patients with H5N1 virus infection have been hospitalized 4–6 days after onset of illness [13, 31, 56, 63, 64] (table 2) In fatal cases, the median time from onset to death was 9 days [63]. At hospital admission, some patients have had sputum production, and the most severely ill patients with H5N1 virus infection have had respiratory distress, tachypnea, rales on auscultation, and evidence of pneumonia [15, 31, 34, 48, 52, 53, 55–57, 64, 65]. In a case series by Liem et al. [64], the mean room air oxygen saturation at hospital admission was 85%. Common laboratory findings at hospital admission include leukopenia, lymphopenia, and mild-to-moderately decreased platelet counts [48, 52, 56, 64]. However, for patients with a clinically mild illness, there was no decrease in the white blood cell count [48, 53]. Chest radiographic findings included

Table 2. Characteristics of hospitalized patients with H5N1 virus infection reported in case series.

Study site (study period) [ref.]	No. of patients	Median age (range), years	Median duration from onset to hospitalization (range), days	Mortality, %	H5N1 virus strain	Findings
Vietnam (2003–2004) [52] ^f	10	12.5 (5–24)	6 (3–8)	80	Clade 1	Signs and symptoms at admission: fever (100% of patients), cough (100%), diarrhea (60%), sputum production (40%), myalgia (0%), conjunctivitis (0%). Laboratory findings at admission: median WBC count, 2100 cells/mm ³ ; median lymphocyte count, 705 cells/mm ³ ; median platelet count: 75,500 platelets/mm ³ . Chest radiograph finding at admission: pneumonia (100%).
Thailand (2004) [55]	12	12 (2–58)	...	67	Clade 1	Signs and symptoms at admission: fever (100% of patients), cough (100%), dyspnea (100%), sore throat (75%), myalgia (42%), diarrhea (42%), rhinorrhea (33%), vomiting (25%), abdominal pain (17%), conjunctivitis (0%). Laboratory findings at admission: median WBC count, 4430 cells/mm ³ ; median lymphocyte count, 1047.5 cells/mm ³ ; median platelet count, 178,000 cells/mm ³ .
Vietnam (2004–2005) [65]	29	35.1 (14–67) ^b	6 (4–8)	24	Clade 1	Significant differences in initial laboratory findings between patients who survived and those who died: Median WBC count, 7800 vs. 3400 cells/mm ³ ; median neutrophil count, 6800 vs. 2300 cells/mm ³ ; median platelet count: 214,000 vs. 86,000 platelets/mm ³ ; median albumin level, 34.5 vs. 21.7 g/L; median aspartate aminotransferase level, 45 vs. 327 U/L; median blood urea nitrogen level, 4.5 vs. 9 mmol/L.
Indonesia (2005) [48] ^c	8	8.5 (1–38)	7 (5–9)	50	Subclade 2.1	Signs and symptoms at admission: feverishness (100% of patients), cough (88%), rhinorrhea (50%), shortness of breath (25%). Laboratory findings at admission: median WBC count, 3590 cells/mm ³ ; median lymphocyte count, 1421 cells/mm ³ ; median platelet count, 220,500 platelets/mm ³ . Chest radiograph finding at admission: bilateral infiltrates (75%).
Turkey (2005–2006) [53]	8	10 (5–15)	8 (1–10)	50	Subclade 2.2	Signs and symptoms at admission: fever (100% of patients), cough (88%), tachypnea (88%), sore throat (75%), myalgia (50%), diarrhea (38%), gum bleeding (38%), headache (12%), rhinorrhea (12%), conjunctivitis (12%). Laboratory findings at admission: median WBC count, 3800 cells/mm ³ ; median lymphocyte count, 1250 cells/mm ³ ; median platelet count, 107,500 platelets/mm ³ ; median lactate dehydrogenase level, 910 U/L; median creatine kinase level, 321 U/L; median D-dimer level, 2.6 mg/dL. Chest radiograph finding at admission: pneumonia (88%).

Vietnam (2004–2006) [64]	67	25 (10–50)	6 (1–20)	39	Clade 1	Signs and symptoms at admission: cough (89% of patients), temperature $\geq 38^{\circ}\text{C}$ (75%), tachypnea (81%), rales on auscultation (82%), mean oxygen saturation with room air $\leq 90\%$, oxygen saturation $\leq 90\%$ (46%), hypotension (29%), required mechanical ventilation (45%). Laboratory findings at admission: median WBC count, 5600 cells/mm ³ ; median lymphocyte count, 1173 cells/mm ³ (with 73% of patients with lymphopenia [i.e., <1500 cells/mm ³]); median platelet count, 174,000 platelets/mm ³ . Chest radiograph findings at admission: infiltrates (84%) and pleural effusion (13%). Significant findings: Patients who died were younger than patients who survived; at admission, patients who died were more likely to have diarrhea, fever, tachycardia, hypotension, hypoxia, leukopenia, lymphopenia, neutropenia, and thrombocytopenia than were patients who survived. There was a higher survival rate with oseltamivir treatment; corticosteroid treatment was associated with increased risk of fatal outcome.
Cambodia (2005–2006) [38]	6	16 (3–28)	6 (2–7)	100	Clade 1	Signs and symptoms at admission: history of feverishness (100% of patients), cough (100%), dyspnea (100%), presence or history of diarrhea (80%), presence or history of abdominal pain (33%), sore throat (17%). Laboratory findings at admission: median WBC count, 4200 cells/mm ³ ; median lymphocyte count, 1000 cells/mm ³ .
Indonesia (2005–2007) [66] ^c	27	16.9 ^b	...	78	Subclade 2.1	Signs and symptoms at admission: median temperature, 37.8°C (range, 35.8°C–40°C); median respiratory rate, 35 breaths/min (range, 15–60 breaths/min). Chest radiograph findings at admission: abnormal findings (74% of patients), pneumonia (70%), pleural effusions (15%).
Indonesia (2006–2008) [31]	127	20 (2–67)	6 (1–16)	81	Subclade 2.1	Early signs and symptoms: fever (93% of patients), cough (32%), rhinorrhea (17%), nausea (13%), dizziness (10%), dyspnea (9%), vomiting (7%), headache (6%), myalgia (1%). Signs and symptoms at admission: fever (99%), cough (88%), dyspnea (84%), rhinorrhea (25%), vomiting (16%), diarrhea (14%). Significant finding: Earlier oseltamivir treatment was associated with survival.
China (2006–2008) [56]	26	29 (6–62)	...	65	Subclade 2.2 (2 patients) and subclade 2.3.4 (24 patients)	Early signs and symptoms: fever (92% of patients), cough (58%), chills (46%), sputum production (35%), malaise (39%), myalgia (42%), headache (15%), rhinorrhea (12%), sore throat (8%), vomiting (8%), abdominal pain (4%), diarrhea (4%). Signs and symptoms at admission: cough (77%), fever (69%), tachypnea (69%), sputum production (58%), chills (46%), malaise (46%), myalgia (31%), dyspnea (23%), headache (15%), sore throat (15%), vomiting (12%), diarrhea (8%), abdominal pain (4%) rhinorrhea (4%). Laboratory findings at admission: median WBC count, 3500 cells/L; median lymphocyte count, 600 cells/L. Chest radiograph findings at admission: all patients had pneumonia (62% with bilateral infiltrates and 38% with unilateral infiltrates). Significant findings: Patients who died had significantly lower nadir platelet counts, a higher median lactate dehydrogenase level, a higher rate of acute respiratory distress syndrome, and higher rate of cardiac failure, compared with survivors.
Vietnam (2007) [15]	8	23 (4–30)	6 (4–6)	63	Subclade 2.3.4	Signs and symptoms at admission: fever (100% of patients), cough (100%), dyspnea (100%), tachypnea (100%), chest pain (88%), diarrhea (38%), chills (38%), vomiting (25%). Laboratory findings at admission: median WBC count, 4100 cell/mm ³ ; median lymphocyte count, 750 cells/mm ³ ; median platelet count, 117,000 platelets/mm ³ ; Chest radiograph findings at admission: pneumonia (100%), bilateral infiltrates (100%).

NOTE. These case series are for cases that occurred since November 2003 and reported as of 15 May 2009. WBC, white blood cell.

^a Some of the data were reported from cases included in Liem et al. [64].

^b Mean age.

^c Some of the data were reported from cases included in Kandun et al. [31].

patchy, interstitial, lobar, and/or diffuse infiltrates, consolidation, pleural effusion, and pneumothorax [15, 34, 48, 52, 53, 55, 56, 64–68]. Respiratory failure is common, and some patients have developed the acute respiratory distress syndrome with very high mortality [15, 55, 56]. A small number of cases showed evidence of secondary bacterial [52, 53], fungal [15, 19], suspected nosocomial bacterial [69], or ventilator-associated pneumonia [15, 39]. In Chotpitayasunondh et al. [55], it was reported that a patient was coinfecting with H5N1 virus and human immunodeficiency virus. It is unknown whether the rarity of reported bacterial complications reflects infrequent bacterial coinfection, inadequate microbiological workup, or the use of broad-spectrum antibiotic therapy.

Extrapulmonary complications due to H5N1 virus infection include cardiac failure [55, 56], renal disease [55, 56], encephalitis [58], multiorgan failure [66], and disseminated intravascular coagulation [56, 70]; these complications occurred in patients with fatal outcomes. Some patients had nonbloody diarrhea, which may have been due to H5N1 virus infection or may have been associated with antibiotic therapy [37–39, 48, 56, 58]; in a study by Liem et al. [64], nonbloody diarrhea was associated with death. The laboratory findings for severely ill patients have included hypoalbuminemia [48] and elevated levels of hepatic transaminases [38, 48, 56], creatinine kinase [53, 56], and lactic dehydrogenase [56]. A poor prognosis was observed among patients with neutropenia and increased levels of alanine aminotransferase at hospital admission [64]. Spontaneous abortions have been reported among pregnant women [13, 56].

PATHOGENESIS OF H5N1 VIRUS INFECTION

Pulmonary pathogenesis. Limited autopsy studies of patients with H5N1 virus infection have identified diffuse alveolar damage [19, 71, 72]. High pharyngeal H5N1 viral replication was correlated with low T lymphocyte counts and high levels of chemokines and cytokines in peripheral blood of critically ill patients [37]. H5N1 viral RNA was detected in respiratory specimens up to 15–16 days after onset of illness [19, 37] and in trachea and lung autopsy specimens 27 days after onset of illness [19]. Elevated levels of proinflammatory cytokines were documented in clinical specimens from patients infected with H5N1 virus, in human primary alveolar and bronchial epithelial cells, and in macrophages in vitro [73–75], although differential expression of cytokines and chemokines in lung tissue from patients who died was reported [76]. Nonhuman primates that were experimentally infected with H5N1 virus had severe lower respiratory disease, with H5N1 virus targeting type II pneumocytes [77, 78] and macrophages [77]. In macaques that were infected with H5N1 virus, severe necrotizing bronchiolitis and alveolitis were noted within 24 hours (with induction and secretion of high levels of interferons and inflammatory cyto-

kines), interleukin (IL)–6 cytokinemia was documented, and disruption of the cell-mediated antiviral response was observed [78].

Additional data suggest how cytokine induction by H5N1 virus damages lung tissues. In 3 fatal cases of H5N1 virus infection, extensive expression of cyclo-oxygenase (COX)–2 was found in bronchial epithelial cells and pneumocytes, but not in alveolar macrophages [75]. However, Lee et al. [75] observed the rapid induction and elevation of proinflammatory cytokines such as tumor necrosis factor (TNF)– α in uninfected lung epithelial cells by soluble factors secreted by H5N1 virus-infected macrophages, which was attenuated by selective COX-2 inhibitors in vitro. Acute lung injury induced by inactivated H5N1 virus was observed in mouse alveolar macrophages, and respiratory oxidative stress induced by inactivated H5N1 virus was observed in human peripheral blood monocytes [79]. In a murine model, inactivated H5N1 virus caused respiratory oxidative stress and the release of oxidized phospholipids, which activated Toll-like receptor 4 and stimulated IL-6 production, leading to inflammation and alveolar damage [79]. High levels of oxidized phospholipids were found in inflammatory exudates that lined the injured air spaces and alveolar macrophages of lungs from H5N1 virus-infected patients with acute respiratory distress syndrome [74]. Mice deficient in IL-6, TNF- α , or the chemokine CCL2 or mice treated with corticosteroids died when infected with H5N1 virus [80]. Apoptosis of human alveolar epithelial cells has been reported [81]. Overall, these studies appear to implicate high and prolonged viral replication and the induction of high levels of proinflammatory cytokines in the pathogenesis of acute lung injury.

Extrapulmonary pathogenesis. Some complications may be related to H5N1 virus infection outside of the respiratory tract. H5N1 viral RNA was detected in or virus was recovered from blood [37], serum [58], or plasma [82] specimens from severely ill patients who died. Nine fatal cases of H5N1 virus infection—in which H5N1 viral RNA was detected in blood during critical illness—had higher pharyngeal viral loads than did 5 nonfatal cases of H5N1 virus infection and 6 nonfatal cases of seasonal influenza in patients without detectable viral RNA in the blood [37]. H5N1 virus was isolated from a cerebrospinal fluid specimen obtained from a pediatric patient with encephalitis who had died [58]. Reactive hemophagocytosis with lymphoid depletion was reported [19, 83]. H5N1 viral antigen, RNA, or nucleic acid was detected in extrapulmonary tissue specimens (e.g., lymph node T cells, cerebral neurons and astrocytes, the small and large intestines, and bone marrow) obtained from a small number of patients who had died [19, 84]. Among critically ill patients who later died, H5N1 viral RNA was detected in rectal swab specimens or diarrheal stool specimens, or H5N1 virus was isolated from these specimens [37, 38]. Vertical transmission was documented in a

pregnant woman, with infection of placenta, fetal lung, liver macrophages, and mononuclear cells [19]. H5N1 viral infection of placental neutrophils was reported, suggesting a mechanism for viral dissemination [85]. Thus, in critically ill patients with fatal outcomes, extrapulmonary H5N1 viral dissemination, including viremia, can occur.

Intranasal inoculation of mice with clade 1 or subclade 2.2 H5N1 virus caused respiratory, brain, and gastrointestinal tract infections, whereas only intragastric inoculation with subclade 2.2 virus produced systemic infection [86]. In ferrets, intranasal inoculation with subclade 2.3 or 2.1 virus produced very mild disease or no infection, respectively, and consumption of chicken meat infected with subclade 2.3 virus produced mild respiratory disease [86]. These and other animal data suggest differences in pathogenicity and site of infection by route of exposure, H5N1 virus clade and/or subclade, as well as multiple viral gene factors [86–91].

CLINICAL MANAGEMENT AND TREATMENT OF PATIENTS

In most cases, the clinical management of patients consists of antiviral treatment and supportive care for complications. Only general guidelines exist for the clinical management of severely ill patients with H5N1 virus infection in intensive care units [92]. No detailed data on the clinical management of severely ill patients with H5N1 virus infection are available, and only very limited data on the clinical course of patients with H5N1 virus infection have been published [56, 64]. The WHO recommends that H5N1 virus-infected patients with acute respiratory distress syndrome be managed similarly to patients with acute respiratory distress syndrome of other etiologies (i.e., with the use of lung-protective strategies, including low tidal volume ventilation) [92]. The WHO has proposed collection of a minimal clinical data set [93], and a standard data collection instrument that includes epidemiological and clinical data was proposed [94].

Antiviral treatment. In addition to supportive care and ensuring adequate oxygenation [92], oral antiviral treatment with the neuraminidase inhibitor oseltamivir is recommended [13, 92, 95]. In a small study by de Jong et al. [96], clade 1 H5N1 viral levels were observed to decrease substantially in 4 surviving patients after 3–5 days of oseltamivir treatment. However, oseltamivir resistance developed in other patients during treatment, including 1 patient treated early (i.e., beginning on the second day of illness) [96]. The optimal dose and duration of oseltamivir treatment in H5N1 patients are unknown. The WHO recommends consideration of higher oseltamivir doses and a longer duration of treatment for patients with severe disease or diarrhea and for patients with late clinical presentation [13, 92]. The administration of oseltamivir via a naso-

gastric tube for patients who receive mechanical ventilation can achieve sufficient drug levels [97].

Limited data from Egypt [13] and Indonesia [31] suggest that early treatment with oseltamivir may prevent severe H5N1 virus infection and improve survival, and other data suggest that antiviral treatment may be beneficial, compared with no treatment [13, 64]. In China, receipt of any antiviral medication was associated with survival [56]. However, analyses are needed that control for severity of illness, other treatments, H5N1 virus clade and/or subclade, and the timing of antiviral treatment for a large number of patients. Data from oseltamivir clinical treatment trials in patients with H5N1 virus infection are unavailable. Because the pathogenesis of H5N1 virus infection appears to be driven by high viral replication that triggers cytokine dysregulation [37, 98], and because most patients do not receive a diagnosis of H5N1 virus infection until late in their clinical course [62], antiviral therapy alone may not be beneficial.

Antiviral resistance. Antiviral resistance in H5N1 virus strains may exist prior to treatment or can develop during treatment. Oseltamivir resistance in clade 1 H5N1 virus variants associated with a specific H274Y mutation in the neuraminidase gene was reported during treatment of 2 patients who died [96] and was detected in subpopulations of strains recovered from samples obtained from a surviving patient who received oseltamivir chemoprophylaxis [99]. Oseltamivir resistance associated with a different neuraminidase gene mutation (N294S) was reported during and before treatment among patients infected with clade 1 and subclade 2.2 virus strains, respectively [99, 100]. In vitro data suggest that the H274Y mutation confers relatively high oseltamivir resistance, compared with intermediate resistance conferred by the N294S mutation [101]. Most clade 1 and subclade 2.1 viruses are resistant to the adamantanes (amantadine and rimantadine) [8]. Clade 1 virus strains resistant to oseltamivir retained susceptibility to the neuraminidase inhibitor zanamivir [78, 79]. H5N1 virus strains circulating among birds can exhibit variable susceptibilities to oseltamivir over time and across clades and subclades [102, 103]. An up to 8-fold reduced oseltamivir susceptibility in vitro was reported in subclade 2.3.4 viruses, compared with the highly susceptible clade 1 virus strains isolated from patients in Vietnam before treatment [15]. These subclade 2.3.4 viruses demonstrated reduced susceptibility to zanamivir but remained sensitive to amantadine and rimantadine [15].

The clinical significance of in vitro oseltamivir resistance identified in H5N1 virus strains is unknown, including whether higher dosing of oseltamivir has clinical effectiveness in patients infected with strains with intermediate resistance. However, these data suggest that combination antiviral treatment with a neuraminidase inhibitor and an adamantane drug [104] or other combinations (oseltamivir-ribavirin) [105] for subclade

2.2 and 2.3.4 virus infections should be strongly considered. Combination antiviral treatment has been administered to some patients with H5N1 virus infection, although data are limited [56]. Triple therapy (amantadine, ribavirin, and oseltamivir) may suggest a strategy for antiviral resistance [106]. Animal data suggest a benefit of treatment with intramuscular peramivir [107], intravenous zanamivir [108], or type 1 interferons [109, 110], but no data on patients with H5N1 virus infection are available for these drugs. Other strategies that target viral endonuclease or viral RNA polymerase have been proposed [111].

Immunomodulator and anti-inflammatory agents. Although cytokine dysregulation is believed to be a major factor in acute lung injury and in the pathogenesis of H5N1 virus infection, the role of immunomodulator therapy for patients with H5N1 virus infection is unclear. No benefit has been identified for the use of corticosteroids, and the WHO recommends against administering corticosteroids except for septic shock with adrenal insufficiency [13, 92]. Zanamivir treatment combined with anti-inflammatory therapy, including COX-2 inhibitors, of clade 1 H5N1 virus-infected mice resulted in a higher survival, compared with the use of monotherapy [112], but data are unavailable on combined antiviral and anti-inflammatory therapy for patients with H5N1 virus infection. Aspirin should not be administered to H5N1 virus-infected patients aged <18 years because of the risk of Reye syndrome [2, 39]. Use of generic anti-inflammatory or immunomodulator therapy (statins, fibrates, or chloroquine) during the next pandemic has been proposed [113], but their effectiveness in treating patients with H5N1 virus infection is unknown.

Immunotherapy. A small number of severely ill patients with H5N1 virus infection in China received passive immunotherapy. Two patients with respiratory failure who required invasive mechanical ventilation received transfused convalescent serum samples from H5N1 virus-infected patients who survived, and both recipients recovered [56, 114]. A third patient with subclade 2.3.4 H5N1 virus infection and with pneumonia requiring positive-pressure ventilation received convalescent plasma from a participant in a clade 1 H5N1 vaccine clinical trial and also made a full recovery [34]. Given the small number of patients and the uncontrolled use of immunotherapy and other coadministered therapies, including antivirals, the effectiveness of convalescent plasma therapy among patients with H5N1 virus infection is unclear. Nevertheless, such results should stimulate further investigations of immunotherapy for H5N1 virus-infected patients with clinical and virological outcomes.

Another approach is the production of monoclonal antibodies that neutralize H5N1 virus strains using different sources, and some studies reported evidence of broad cross-clade activity [115–127]. H5-specific neutralizing antibodies

were observed to have therapeutic and prophylactic benefits in H5N1 virus-infected mice [116], including efficacy when administered up to 5 days after H5N1 virus infection, compared with control mice [121], and after extrapulmonary H5N1 virus dissemination had occurred [122]. Sui et al. [120] identified high-affinity human antibodies that targeted the postviral attachment fusion process and that demonstrated high efficacy in vitro and in vivo against H5N1 and seasonal influenza A (H1N1) viruses. Overall, these findings suggest the potential for targeted monoclonal or polyclonal antibody therapy with broad neutralizing ability for human H5N1 virus infection, including against strains of different virus clades and subclades.

INFECTION CONTROL ISSUES

Nosocomial H5N1 virus transmission from patients to family members [32, 34] and to a health care worker [128] was reported after prolonged, close, unprotected contact with a severely ill patient, and serological evidence of patient-to-health care worker transmission was reported [129]. Patients with suspected, probable, or confirmed H5N1 virus infection should be isolated in single rooms with designated caregivers. Respiratory secretions, body fluids, stool, and all other clinical specimens should be considered potentially infectious, although the detection of H5N1 virus in urine has not been reported. The WHO recommends use of personal protective equipment (gown, gloves, goggles, and surgical mask) and implementation of standard, contact, and droplet precautions for routine care of patients with H5N1 virus infection [92, 130, 131]. For aerosol-generating procedures (suctioning, intubation, or administering aerosolized bronchodilators), the WHO recommends using a fit-tested N95-equivalent or higher particle-filtering respirator [91, 130, 131], whereas the CDC recommends implementation of airborne precautions, if available, for all patients suspected of having H5N1 virus infection in the United States [132]. Aerosol-generating procedures should be performed in a single ventilated room with at least 12 air exchanges per hour or in a negative pressure room, if available [92]. It is extremely important to educate health care workers and family member caregivers about H5N1 virus transmission and infection control and to provide them with personal protective equipment, given the transmission of infection to blood-related family members described in past studies [32, 34]. H5N1 vaccine is unavailable outside of clinical trials, but annual influenza vaccination is recommended for health care workers to prevent seasonal influenza. Oseltamivir chemoprophylaxis of H5N1 virus-exposed persons is recommended, and the WHO issued guidelines for prioritization depending on antiviral availability [95]. Close monitoring of family members, health care workers, and others who have had close contact with H5N1 virus-infected patients is needed.

CONCLUSIONS

To date, there is a paucity of detailed clinical data and available information on optimal evidence-based clinical management of patients with H5N1 virus infection, and much more clinical research is needed. The systematic collection, analysis, and publication of detailed clinical data are needed to standardize and improve clinical care of H5N1 virus-infected patients worldwide and especially to guide intensive care management. Given that sporadic human infections with H5N1 virus are expected to continue among exposed persons, many challenges remain, including the ability to detect and treat H5N1 virus infection early. The development and availability of highly accurate, specific, simple, rapid, and inexpensive point-of-care H5N1 diagnostic tests are needed. Comprehensive data on clinically mild cases and additional autopsy data will help further understanding of the pathogenesis of H5N1 virus infection.

Although the pathogenesis of human H5N1 virus infection is not completely understood, data suggest that controlling viral replication and cytokine dysregulation should be targeted. Given the high mortality for patients with H5N1 virus infection and lack of definitive treatment to date, there is an urgency to consider and assess new treatment strategies, including combination antiviral treatment (oral, inhaled, or parenteral administration and higher dosing) with anti-inflammatory agents and immunotherapy, and to make any effective therapies widely available.

Acknowledgments

Potential conflicts of interest. T.M.U.: no conflicts.

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