REVIEW ARTICLE

CURRENT CONCEPTS

Update on Avian Influenza A (H5N1) Virus Infection in Humans

Writing Committee of the Second World Health Organization Consultation on Clinical Aspects of Human Infection with Avian Influenza A (H5N1) Virus*

THE UNPRECEDENTED EPIZOOTIC OF AVIAN INFLUENZA A (H5N1) VIRUSES among birds continues to cause human disease with high mortality and to pose the threat of a pandemic. This review updates a 2005 report[1] and incorporates information recently published or presented at the Second World Health Organization (WHO) Consultation on Clinical Aspects of Human Infection with Avian Influenza A (H5N1) Virus.[2]

VIRAL ECOLOGY

Highly pathogenic avian influenza A (H5N1) viruses are entrenched among poultry in parts of Asia, Africa, and perhaps the Middle East. The highly pathogenic avian influenza H5 hemagglutinin has evolved into many phylogenetically distinct clades and subclades (Fig. 1)[4,5] that generally correlate with antigenic differences that must be considered in the selection of candidates for H5N1 vaccines.[6,7] These diverse lineages have been largely separate geographically since 2005 (Fig. 1),[5] although clade 2.3 viruses from China have recently circulated in other Southeast Asian countries.[8]

The influenza A (H5N1) viruses that have infected humans have been entirely avian in origin, and they reflect strains circulating locally among poultry and wild birds. Avian influenza viruses can be maintained, amplified, and disseminated in live-poultry markets. Migratory birds may spread A (H5N1) viruses to new geographic regions, but their importance as an ecologic reservoir is uncertain. The spread of influenza A (H5N1) viruses appears to be principally related to the movement of poultry and poultry products,[9,10] although recent outbreaks of clade 2.2 virus infection in sub-Saharan Africa,[11] Egypt, and Europe may indicate introduction of the virus by wild birds. The risk of the introduction of influenza A (H5N1) viruses into North America by birds migrating through Alaska appears to be low.[12]

EPIDEMIOLOGY OF HUMAN INFECTIONS

INCIDENCE AND DEMOGRAPHIC CHARACTERISTICS

Despite widespread exposures to poultry infected with avian influenza A (H5N1) viruses,[13,14] influenza A (H5N1) disease in humans remains very rare. Since May 2005, the numbers of both affected countries[13] and confirmed cases of influenza A (H5N1) virus infection (340 cases as of December 14, 2007) have increased, in part because of the spread of clade 2.2 viruses across Eurasia and to Africa[5,15] (Fig. 1 of the Supplementary Appendix, available with the full text of this article at www.nejm.org).

The median age of patients with influenza A (H5N1) virus infection is approximately 18 years, with 90% of patients 40 years of age or younger and older adults

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underrepresented.[16] The overall case fatality proportion is 61%; it is highest among persons 10 to 19 years of age and lowest among persons 50 years of age or older.[16] Whether preexisting immunity, differences in exposure, or other factors might contribute to the apparently lower frequency of infection and lethal illness among older adults is uncertain. Most patients with influenza A (H5N1) virus infection were previously healthy. Of six affected pregnant women, four have died, and the two survivors had a spontaneous abortion.[17]

Increases in human cases of influenza A (H5N1) have been observed during cooler months in association with increases in outbreaks among poultry (see Fig. 1 of the Supplementary Appendix).[18] However, because cases have occurred year-round, clinicians must be alert to possible human infection at any time, especially in countries with outbreaks of influenza A (H5N1) among birds. To date, no cases of influenza A (H5N1) illness have been identified among short-term travelers visiting countries affected by outbreaks among poultry or wild birds,[19] although clinicians in unaffected countries should consider this possibility in travelers with exposures to poultry.

Surveillance for cases of influenza A (H5N1) has focused on patients with severe illness, but milder illnesses in children, which are not pneumonic,[20,21] occur. Limited seroepidemiologic studies conducted since 2003 involving villagers living with backyard poultry, workers in live-poultry markets, and health care workers suggest that asymptomatic or mild human influenza A (H5N1) virus infection is rare (Table 1 of the Supplementary Appendix).[14]

Figure 1 (facing page). Evolution of the Hemagglutinin and Other Key Mutations Associated with Virulence or Drug Resistance in Avian Influenza A (H5N1) Virus.

The phylogenetic tree is for the hemagglutinin gene of highly pathogenic avian influenza A (H5N1) viruses. The geographic distributions refer to avian isolates, and the tree is based on publicly available sequences. Clade 0 includes viruses that were first recognized to cause human infections in Hong Kong Special Administrative Region in 1997. Viruses from clades and subclades 0, 1, 2.1, 2.2, 2.3, and 7 have caused human disease. Clade 1 viruses predominated in Vietnam, Thailand, and Cambodia in the early phase of the outbreak (2004–2005), and clade 2.1 viruses are endemic in Indonesia. Clade 2.2 viruses were associated with a major outbreak of H5N1 disease in migratory birds in Qinghai Lake, China, and have since spread, causing avian disease in Central and South Asia, the Middle East, Europe, and Africa and human disease in western Asia, the Middle East, and Africa. Clade 2.3 has become dominant in southern China and has also been detected in adjacent countries. (Modified from the WHO Web site: www.who.int/csr/disease/avian_influenza/guidelines/nomenclature/en/index.html.) The influenza genome contains eight individual segments of RNA, several of which encode two proteins. Within clade 1 or clade 2.1 viruses, polymerase basic protein 2 (PB2) Glu627Lys is observed in some isolates of human viruses but not in avian viruses.[3] Some human clade 1 viruses without PB2 627Lys have PB2 701Asn; clade 2.2 viruses of both human and avian origin have PB2 Glu627Lys.[4] The importance of sequence variations in NS1, in which most influenza A (H5N1) viruses contain a carboxyl-terminus–sequence motif that mediates binding to various cellular proteins bearing a PDZ domain, remains to be determined.

TRANSMISSION

Direct avian-to-human H5N1 virus transmission is the predominant means of human infection, although the exact mode and sites of influenza A (H5N1) virus acquisition in the respiratory tract are incompletely understood. Handling of sick or dead poultry during the week before the onset of illness is the most commonly recognized risk factor.[22,23] Most patients have acquired A (H5N1) infection from poultry raised inside or outside their houses. Slaughtering, defeathering, or preparing sick poultry for cooking; playing with or holding diseased or dead poultry; handling fighting cocks or ducks that appear to be well; and consuming raw or undercooked poultry or poultry products have all been implicated as potential risk factors.[21-24] The defeathering of dead wild swans was implicated in one case cluster.[25]

The influenza A (H5N1) virus can also infect multiple mammalian hosts,[26,27] including domestic cats[28] and dogs.[29] None have been implicated in influenza A (H5N1) virus transmission to humans yet, but any animal infected with the virus theoretically poses a risk of transmission and of being a host for viral adaptation to mammals.[26]

Clusters of human influenza A (H5N1) illness with at least two epidemiologically linked cases have been identified in 10 countries and have accounted for approximately one quarter of cases.[20,21,24,30-32] Most clusters have involved two or three persons; the largest affected eight. More than 90% of case clusters have occurred among blood-related family members, suggesting possible genetic susceptibility, although one statistical model indicated that these clusters might have occurred because of chance alone.[33] Most persons

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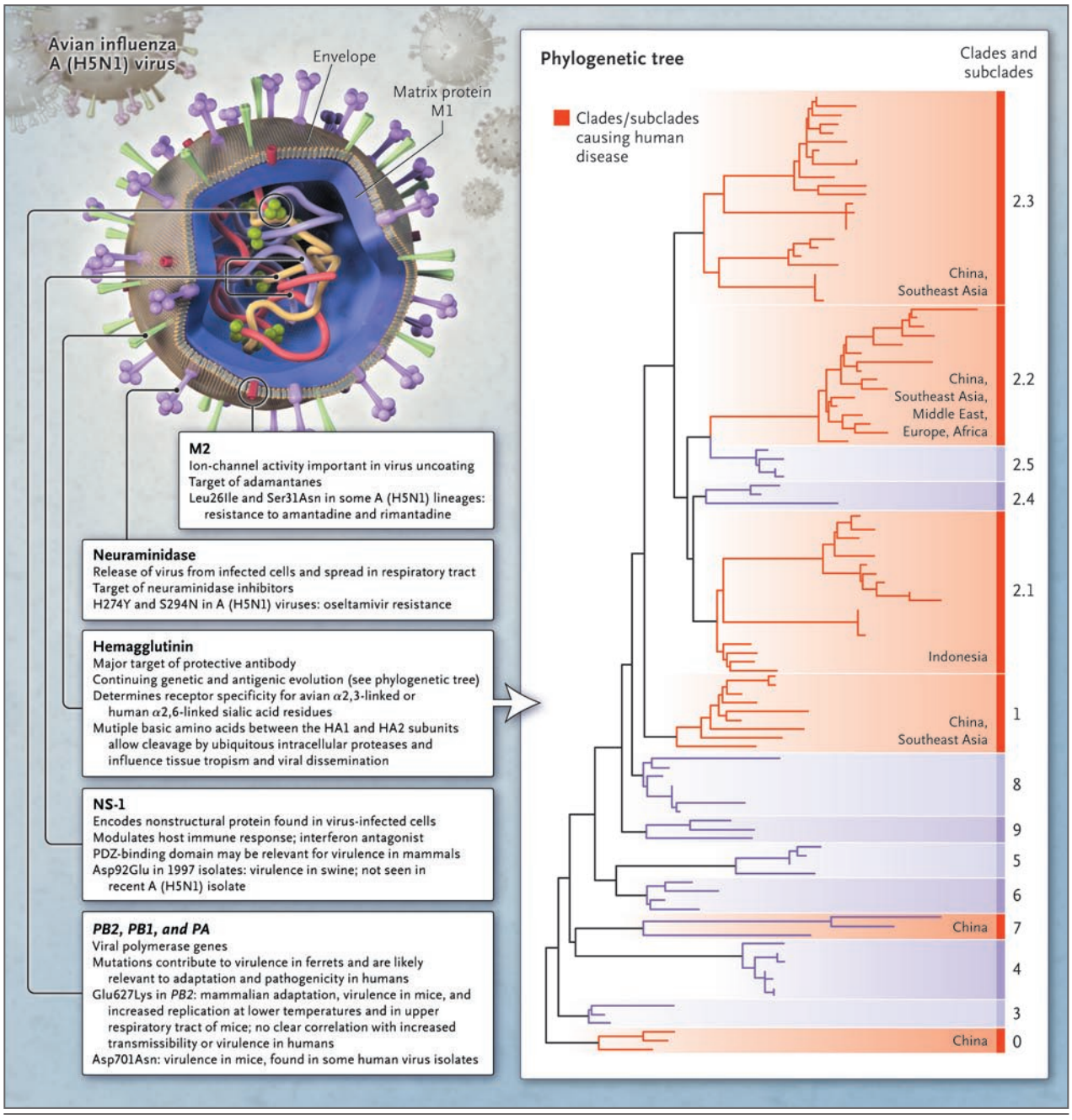

in case clusters probably acquired infection from common-source exposures to poultry, but limited, nonsustained human-to-human transmission has probably occurred during very close, unprotected contact with a severely ill patient.[20,30,32] In the largest cluster, transmission probably occurred from the index case to six blood-related family members and subsequently to another family member.[32] Respiratory secretions and all bodily fluids, including feces, should be considered potentially infectious.

In one quarter or more of patients with influenza A (H5N1) virus infection, the source of exposure is unclear, and environment-to-human transmission remains possible.[20,24] For some patients, the only identified risk factor was visiting a live-poultry market.[34,35] Plausible transmission routes include contact with virus-contaminated

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fomites or with fertilizer containing poultry feces, followed by self-inoculation of the respiratory tract or inhalation of aerosolized infectious excreta. It is unknown whether influenza A (H5N1) virus infection can begin in the human gastrointestinal tract. In several patients, diarrheal disease preceded respiratory symptoms,[36] and virus has been detected in feces.[3,37] Acquisition of influenza A (H5N1) virus infection in the gastrointestinal tract has been implicated in other mammals.[26] Drinking potable water and eating properly cooked foods are not considered to be risk factors, but ingestion of virus-contaminated products or swimming or bathing in virus-contaminated water might pose a risk.

INCUBATION PERIOD

After exposure to infected poultry, the incubation period generally appears to be 7 days or less, and in many cases this period is 2 to 5 days. In clusters in which limited, human-to-human transmission has probably occurred, the incubation period appears to be approximately 3 to 5 days, although in one cluster it was estimated to be 8 to 9 days.[20,30]

PATHOGENESIS

VIRAL FACTORS

The viral and host factors that determine host restriction and disease manifestations are incompletely understood.[38] Preferential binding of the influenza A (H5N1) virus to α2,3-linked sialic acid receptors on avian cells[39] is thought to be key in preventing influenza A (H5N1) and other avian influenza viruses from readily infecting humans. Some influenza A (H5N1) viruses isolated from humans have acquired mutations that permit binding to both α2,3-linked sialic acid receptors and α2,6-linked sialic acid receptors,[40] but these mutations appear to be insufficient for efficient human-to-human transmission. To date, influenza A (H5N1) viruses have shown no transmissibility or poor transmissibility between ferrets and between swine, and reassortment between an influenza A (H5N1) virus and an influenza A (H3N2) virus did not confer transmissibility in ferrets.[41] Changes in multiple viral genes are probably required to generate a potentially pandemic influenza A (H5N1) virus.

All recent influenza A (H5N1) viruses retain a polybasic amino acid motif at the HA1–HA2 connecting peptide that is characteristic of highly pathogenic avian influenza viruses. Geographic variations in this motif have not been associated with obvious changes in the virulence of infection in humans. Amino acid substitutions in the polymerase basic protein 2 (*PB2*) gene are associated with mammalian adaptation, virulence in mice, and replication at temperatures present in the upper respiratory tract (Fig. 1).[42] However, these mutations do not correlate with obvious differences in mortality among humans with this viral infection.[3,21]

VIRAL REPLICATION

The primary pathologic process that causes death is fulminant viral pneumonia. The target cells for replication of the influenza A (H5N1) virus include type 2 alveolar pneumocytes and macrophages.[17,43,44] Bronchiolar and alveolar cells, but not epithelia from the trachea or upper respiratory tract, express detectable α2,3-linked sialic acid receptors.[43-45] However, influenza A (H5N1) viruses replicate in ex vivo organ cultures of the upper respiratory tract,[44] postmortem studies show virus in tracheal epithelia,[17,46] and high titers of virus are detectable in specimens of throat and tracheal aspirates from humans infected with influenza A (H5N1) virus.[3] These findings suggest that the initial infection may occur in either the upper or lower respiratory tract, although the latter may support more efficient replication.

Limited data show that patients with influenza A (H5N1) disease may have detectable viral RNA in the respiratory tract for up to 3 weeks, presumably because of negligible preexisting immunity and possibly viral evasion of immune responses.[3] One patient with fatal infection treated with both antiviral agents and corticosteroids had viral antigen and RNA in tracheal samples on day 27 after the onset of illness.[17] Viral loads in the pharynx are higher and plasma viral RNA is detected more often in patients with fatal disease than in those with nonfatal disease, indicating that levels of viral replication influence the outcome.[3] The reported presence of infectious virus in the blood, cerebrospinal fluid, or viscera of several patients with fatal disease indicates that, as in birds and several mammalian species, disseminated infection occurs in some humans.[3,17,36,37,46] A fatal influenza A (H5N1) infection in one pregnant woman who received corticosteroids for treatment of the disease was associated with virus infection of the brain, placenta, and fetus.[17] Infectious virus and viral RNA have been detected

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in feces and intestines, suggesting that the virus sometimes replicates in the gastrointestinal tract.[1,3,36,37,46]

PATHOLOGICAL FINDINGS

The few reported autopsies of patients with influenza A (H5N1) virus infection have shown diffuse alveolar damage with hyaline membrane formation, patchy interstitial lymphoplasmacytic infiltrates, bronchiolitis with squamous metaplasia, and pulmonary congestion with varying degrees of hemorrhage.[17,46,47] Acute exudative, diffuse alveolar damage with macrophages, neutrophils, and activated lymphocytes has been detected in patients who died within 2 weeks after the onset of illness. Apoptosis in alveolar cells and infiltrating leukocytes are prominent findings.[46] Lymphocyte depletion occurs in the spleen, lymph nodes, and tonsils; histiocytic hyperplasia and reactive hemophagocytosis presumably result from host cytokine responses and viral infection. Edema and degeneration of myocytes in the heart and extensive acute tubular necrosis in the kidney have been observed.

HOST RESPONSES

Higher plasma levels of macrophage and neutrophil-attractant chemokines and both proinflammatory and antiinflammatory cytokines (interleukin-6, interleukin-10, and interferon-γ) have been observed in patients with influenza A (H5N1) virus infection — particularly in patients with fatal infection — than in patients with conventional influenza.[3] Plasma levels of cytokines and chemokines correlate positively with pharyngeal viral loads,[3] suggesting that these responses are driven by high-level viral replication. In vitro experiments involving primary human macrophages and lung pneumocytes show differential up-regulation of multiple cytokines by influenza A (H5N1) virus as compared with human influenza viruses,[48] indicating that viral hyperinduction probably contributes to hypercytokinemia.

In mouse models of influenza A (H5N1) virus infection, mice with deficient induction of interleukin-6, macrophage inflammatory protein 1α, or tumor necrosis factor α or its receptors[49,50] and mice treated with glucocorticoids,[50] had similar mortality as compared with wild-type animals; mice without interleukin-1 receptors had increased mortality.[49] Tissue damage in human influenza A (H5N1) disease probably results from the combined effects of unrestrained viral infection and inflammatory responses induced by influenza A (H5N1) infection. Knowledge of the mechanisms of hypercytokinemia is insufficient to guide safe, rational immunomodulatory treatment at present.

CLINICAL FEATURES

Currently, illness due to influenza A (H5N1) viruses typically manifests as severe pneumonia that often progresses rapidly to the acute respiratory distress syndrome. The time from the onset of illness to presentation (median, 4 days) or to

Table 1. Case Fatality Proportion According to Clade or Subclade and Median Time from Onset of Illness to Hospitalization or Death in Patients with Confirmed Influenza A (H5N1) Illness.

Country	Predominant Clade or Subclade*	Case Fatality Proportion	Onset of Illness to Hospitalization		Onset of Illness to Death	
		no. of patients/ total no. (%)	*days*	*no. of patients*	*days*	*no. of patients*
Cambodia, Thailand, Vietnam†	1	66/123 (54)	4	109	9	65
Indonesia	2.1	76/96 (79)	5	64	9	72
Azerbaijan, Djibouti, Egypt, Iraq, Nigeria, Turkey	2.2	26/59 (44)	3	36	9	24
China, Laos	2.3	17/26 (65)	5	16	10	17

* The presumed clade or subclade assignment is based on the known geographic distribution of the viruses and is not verified by individual patient data. Few sequences are available for human isolates in the public database for some countries. Multiple clades and subclades have circulated in China in poultry. The numbers of patients for whom data were available are listed. The analysis was provided by Dr. Christoph Steffen and Dr. Julia Fitzner, WHO, Geneva.

† Among 61 patients with documented clade 1 infection, the case fatality proportion was 75%; the median time from the onset of illness to hospitalization was 5 days in 48 patients, and the median time from the onset of illness to death was 9 days in 46 patients.

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death (median, 9 to 10 days) has remained unchanged from 2003 through 2006 (Table 1).[16] Observed differences in mortality among patients with presumed clade 1 and clade 2 virus infections (Tables 1 and 2)[1,21,24,35,51] are difficult to interpret because of variations in medical practices and the time from the onset of illness to treatment among affected countries.

Febrile upper respiratory illnesses without pneumonia in children have been reported more frequently since 2005.[20,21] Early consultation and antiviral therapy may have altered the clinical course of these illnesses. Less frequent gastrointestinal symptoms have been reported since 2005 (Table 2), suggesting that some manifestations of clade 1 and 2 virus infections may differ from each other. Leukopenia, lymphopenia, mild-to-moderate thrombocytopenia, and elevated levels of aminotransferases are common but not universal (Table 2). Lymphopenia and increased levels of lactate dehydrogenase at presentation have been associated with a poor prognosis.[1,3,21,37] Other reported abnormalities include elevated levels of creatine phosphokinase, hypoalbuminemia, and increased D-dimer levels and changes indicative of disseminated intravascular coagulopathy.[20,21]

The nonspecific clinical presentation of influenza A (H5N1) disease has often resulted in mis-

Table 2. Clinical and Common Laboratory Features of Influenza A (H5N1) Disease at Hospital Admission.*

Variable	Vietnam, Thailand, Cambodia, 2004–2005, Clade 1†	Indonesia, 2005–2006, Clade 2.1‡	China, 2005–2006, Clade 2.3§	Egypt, 2006–2007, Clade 2.2¶	Turkey, Azerbaijan, 2006, Clade 2.2‖
Age — yr					
Median	14–22	18.5	30	12.5	16.5–10.0
Range	2–58	1.5–45.0	12–41	1–75	5–20
Male sex — no./total no. (%)	19/41 (46)	33/54 (61)	3/8 (38)	12/38 (32)	9/16 (56)
Contact with poultry within previous 2 weeks — no./ total no. (%)	31/36 (86)	41/54 (76)	8/8 (100)**	31/38 (82)	8/8 (100)††
Time from onset of symptoms to hospitalization — days					
Median	6–8	5	6	3	5–6
Range	3–8	1–14	3–11	0–14	1–12
Clinical presentation — no./ total no. (%)					
Fever	41/41 (100)	54/54 (100)	8/8 (100)	34/38 (89)	15/16 (94)
Dyspnea	33/37 (89)	51/54 (94)	4/8 (50)	14/38 (37)	7/16 (44)
Cough	40/41 (98)	50/54 (93)	7/8 (88)	27/38 (71)	12/15 (80)
Pneumonia	41/41 (100)	54/54 (100)	8/8 (100)	23/38 (61)‡‡	14/16 (88)
Coryza	9/27 (33)	NR	NR	NR	2/14 (14)
Sore throat	13/41 (32)	NR	NR	26/38 (68)	14/16 (88)
Vomiting	5/31 (16)	6/54 (11)	NR	3/37 (8)	0/7 (0)
Diarrhea	16/31 (52)	6/54 (11)	NR	2/37 (5)	4/14 (29)
Depressed consciousness	NR	NR	NR	3/38 (8)	4/8 (50)
Seizures	NR	1/54 (2)	NR	NR	2/7 (29)
Headache	5/14 (36)	7/54 (13)	NR	19/38 (50)	7/15 (47)
Conjunctivitis	0/22 (0)	NR	NR	14/38 (37)	1/8 (12)
Myalgia	11/37(30)	7/54 (13)	NR	17/38 (45)	4/15 (27)
Leukopenia	17/22 (77)	41/49 (84)	NR	10/37 (27)	11/15 (73)
Lymphopenia	16/24 (67)	16/29 (55)	NR	4/25 (16)	7/13 (54)
Thrombocytopenia	13/24 (54)	29/45 (64)	NR	8/26 (31)	9/13 (69)
Increased aminotransferase levels	20/28 (71)	NR	NR	15/27 (56)	6/8 (75)

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Table 2. (Continued.)

Variable	Vietnam, Thailand, Cambodia, 2004–2005, Clade 1†	Indonesia, 2005–2006, Clade 2.1‡	China, 2005–2006, Clade 2.3§	Egypt, 2006–2007, Clade 2.2¶	Turkey, Azerbaijan, 2006, Clade 2.2‖
Deaths — no./ total no. (%)	32/41 (78)	41/54 (76)	7/8 (88)	15/38 (39)	9/16 (56)
Time from onset of symptoms to death — days					
Median	8–12	9	9	11.5	10–13
Range	4–30	5–19	8–19	6–32	9–17

* The presumed clade or subclade assignment is based on the known geographic distribution of the viruses and is not verified by individual patient data. Few sequences are available for human isolates in the public database for some countries. Multiple clades and subclades have circulated in China in poultry. NR denotes not reported.
† Data are from the WHO Writing Committee.[1]
‡ Data are from Sedyaningsih et al.[24]
§ Data are from Yu et al.[35] and Yu et al.[51]
¶ Data are from Abdel-Ghafar A (unpublished data). The lower mortality among Egyptian patients as compared with Indonesian patients in 2006–2007 could be related to the approximately 2-day shorter time to presentation and lower frequency of pneumonia among the Egyptian patients.
‖ Data for Turkey are from Oner et al.[21] Data for Azerbaijan were provided by the Ministry of Health.
** This number includes six of eight patients who visited live-bird markets but did not have known direct exposure to poultry.
†† Only one of eight patients had contact with poultry in Azerbaijan; exposures were to dead swans.
‡‡ Pneumonia did not develop in 2 of 12 adults (17%) and 13 of 26 children (50%) in Egypt.

diagnosis of subsequently confirmed cases (Table 3); influenza A (H5N1) virus infection has been suspected in only a small number of patients. Health care staff should include influenza A (H5N1) virus infection in the differential diagnosis for patients who present with epidemiologic risk factors and unusual courses of illness, especially rapidly progressing pneumonia (see Fig. 2 of the Supplementary Appendix).

LABORATORY DIAGNOSIS

Detection of viral RNA by means of conventional or real-time reverse-transcriptase polymerase chain reaction remains the best method for the initial diagnosis of influenza A (H5N1).[52] These assays can provide results within 4 to 6 hours and can be performed under biosafety level 2 conditions. The genetic variability of influenza A (H5N1) viruses[7,8] calls for frequent updating of primers and probes. Consequently, access to sequences from recent influenza A (H5N1) viral isolates is essential. To detect other influenza A virus infections and reduce false negative results due to mutations in the H5 hemagglutinin gene, a conserved influenza A gene (e.g., matrix or nucleoprotein) should also be targeted.

Diagnostic yields are higher with throat specimens than with nasal swabs because of higher viral loads of influenza A (H5N1) in the throat.[1,3] However, nasal swabs are useful for detecting human influenza viruses, so collection of both specimens is recommended. If they are available, tracheal aspirates have higher viral titers and yields than specimens from the upper respiratory tract.[3] Negative results in single respiratory specimens do not rule out influenza A (H5N1) virus infection,[21] and repeated collection of multiple specimen types is recommended.[52] Previous antiviral treatment may reduce the diagnostic yield. Detection of influenza A (H5N1) viral RNA in feces or blood may provide prognostic information,[3] but it has lower diagnostic sensitivity than influenza A (H5N1) viral RNA in respiratory specimens.

Commercially available rapid assays for influenza-antigen detection have poor clinical sensitivity for the detection of influenza A (H5N1) virus (Table 2 of the Supplementary Appendix),[1,20,21] and they do not differentiate between human and avian subtypes of influenza A viruses. Although rapid antigen tests have similar analytic sensitivity for detecting human and avian influenza A (H5N1) viruses, they require 1000 times higher levels of virus than viral cultures to be positive.[53]

The detection of anti-H5 antibodies is essential for epidemiologic investigations and may provide retrospective diagnostic confirmation in patients. Seroconversion generally occurs 2 to 3 weeks after infection. Microneutralization assays are the most reliable methods for detecting antibodies to

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Table 3. Initial Diagnosis in Patients with Confirmed Influenza A (H5N1) Virus Infection.*

Diagnosis	Indonesia (N=52)	Thailand (N=25)
	number (percent)	
Pneumonia	24 (46)	11 (44)
Dengue virus infection	6 (12)	4 (16)
Typhoid fever	2 (4)	0 (0)
Upper respiratory illness	14 (27)	4 (16)
Avian influenza	6 (12)	2 (8)
Other	0 (0)	4 (16)†

* Data are from Chotpitayasunondh T and Soeroso S (unpublished data).
† Tuberculosis was diagnosed in one patient, diarrhea in one patient, dizziness in one patient, and leptospirosis in one patient.

avian viruses, but they are labor-intensive and require biosafety level 3 facilities and appropriate strains of influenza A (H5N1) viruses. As compared with initial samples, elevations of four times or more or single titers of 1:80 or more in convalescent-phase samples are considered to be diagnostic.[52] Modified nonpathogenic influenza A (H5N1) virus generated by reverse genetics or lentivirus pseudotyped with H5 hemagglutinin[54] may provide alternatives for performing neutralization tests in biosafety level 2 facilities. Hemagglutination-inhibition assays with the use of horse erythrocytes show promising results but require further validation.

TREATMENT

ANTIVIRAL AGENTS

Susceptibility to current antiviral agents varies among circulating strains of influenza A (H5N1) viruses. Clade 1 viruses and most clade 2 viruses from Indonesia are fully resistant to M2 inhibitors, whereas clade 2 viruses from the lineages in other parts of Eurasia and Africa are usually susceptible (Klimov A: personal communication). As compared with influenza A (H5N1) viruses from 1997 or influenza A (H1N1) viruses in vitro,[55] clade 1 viruses generally show enhanced susceptibility to oseltamivir carboxylate, but the high-level replication of some oseltamivir-susceptible strains requires higher doses or more prolonged administration, or both, in animal models.[55,56] Clade 1 viruses appear to be 15 to 30 times more sensitive to oseltamivir than clade 2 isolates from Indonesia and Turkey,[56,57] although the possible clinical relevance of such differences in oseltamivir susceptibility remains to be determined. During oseltamivir therapy, the emergence of highly resistant variants with an H274Y neuraminidase mutation may be associated with a fatal outcome.[58] Infection by influenza A (H5N1) viruses containing an N294S mutation that causes a reduction in oseltamivir susceptibility by a factor of 12 to 15 times was reported to be present in two Egyptian patients with fatal disease before therapy,[59] and avian influenza A (H5N1) viruses with reduced susceptibility to neuraminidase inhibitors are occasionally detected.[60]

Early treatment with oseltamivir is recommended,[61,62] and data from uncontrolled clinical trials suggest that it improves survival (Table 4), although the optimal dose and duration of therapy are uncertain. Mortality remains high despite administration of oseltamivir; late initiation of therapy appears to be a major factor. Uncontrolled viral replication, as reflected in the detection of persistent pharyngeal RNA after completion of standard therapy, is associated with a poor prognosis.[58] Higher levels of viral replication and slower clearance of infection probably occur in the lower respiratory tract.[3] The oral bioavailability of oseltamivir in patients with severe diarrhea or gastrointestinal dysfunction related to influenza A (H5N1) virus infection or those in whom the drug has been administered extemporaneously (e.g., by means of a nasogastric tube) is uncertain.

A higher dose of oseltamivir (e.g., 150 mg twice daily in adults) and an increased duration of therapy, for a total of 10 days, may be reasonable, given the high levels of replication of the influenza A (H5N1) virus, observations of progressive disease despite early administration of standard-dose oseltamivir (75 mg twice daily for 5 days in adults) within 1 to 3 days after the onset of the illness, and the proven safety of higher doses in adults with seasonal influenza, especially if there is pneumonic disease at presentation or evidence of clinical progression.[62] In mouse models of amantadine-sensitive influenza A (H5N1) virus infection, as compared with monotherapy, the combination of oseltamivir and amantadine significantly increased survival rates and inhibited viral replication in the internal organs.[64] No adverse pharmacologic interactions have been shown in humans.[65] In areas where influenza A (H5N1) viruses are likely to be susceptible to amantadine,

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Table 4. Effects of Treatment and Time to Treatment with Oseltamivir on Survival among Patients with Influenza A (H5N1) Infection.*

Type of Infection and Location of Patients	Year	Survival		Days from Onset of Illness to Initiation of Antiviral Therapy		Comment	Reference
				Nonfatal Illness	Fatal Illness		
		no. of survivors/ no. treated (%)	*no. of survivors/ no. not treated (%)*	*median (range)*			
Presumed clade 1 virus infections		45/82 (55)	6/26 (23)			Significant survival benefit with oseltamivir treatment as compared with no treatment (P=0.006, Fisher's exact test)	
Thailand	2004–2005	3/10 (30)	2/7 (29)	5 (4–7)	9 (5–22)		Chotpitayasunondh T (unpublished data)
Vietnam (southern)	2004–2005	5/17 (29)	0/1 (0)	6 (4–12)	5.5 (2–7)		de Jong M (unpublished data)
Vietnam (northern)	2004–2005	37/55 (67)	4/12 (33)	NR	NR	Significant survival benefit with oseltamivir treatment as compared with no treatment (P=0.048); most patients (73%) began to receive oseltamivir after 4 days of illness	Cao T, Thanh Liem N, and Duc Hien N (personal communication)
Cambodia	2005–2006	NA	0/6 (0)			Median time to hospitalization, 6 days (range, 2–7)	Buchy et al.[37]
Presumed clade 2 virus infections		43/106 (41)	1/30 (3)			Significant survival benefit with oseltamivir treatment as compared with no treatment (P<0.001)	
Turkey, clade 2.2 virus infections	2005	4/7 (57)	0/1 (0)	4 (1–10)	8 (8–10)		Oner et al.[21]
Egypt, clade 2.2 virus infections	2006–2007	20/34 (59)	NA	1 (0–3)	4 (1–14)	Significantly shorter time from onset of illness to oseltamivir treatment among patients who survived than among those who did not survive (P=0.001, Kruskal–Wallis test)	Abdel-Ghafar A (unpublished data)
Indonesia, clade 2.1 virus infections	2005–2007	19/65 (29)	1/29 (3)	NR	NR	Significant survival benefit with oseltamivir treatment as compared with no treatment (P=0.005)	Sedyaningsih et al.[63]
Total		88/188 (47)	7/56 (12)			Significant overall survival benefit with oseltamivir treatment as compared with no treatment (P<0.001)	

* NA denotes not applicable, and NR not reported.

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combination treatment with oseltamivir would be reasonable, especially in seriously ill patients.

Although zanamivir is active against oseltamivir-resistant variants with N1 neuraminidase mutations at H274Y[66] or N294S, the value of inhaled zanamivir has not been studied in human influenza A (H5N1) disease. Suboptimal delivery to sites of infection in patients with pneumonic or extrapulmonary disease is a concern. Parenteral delivery of zanamivir or the neuraminidase inhibitor peramivir results in antiviral activity in animal models of influenza A (H5N1) virus infection; these agents and others are under clinical development (Table 3 of the Supplementary Appendix).

OTHER TREATMENTS

Supportive care with correction of hypoxemia and treatment of nosocomial complications remains fundamental in the management of influenza A (H5N1) disease.[2,62] Corticosteroids should not be used routinely.[62] Corticosteroid therapy has thus far not been shown to be effective in patients with influenza A (H5N1) virus infection,[1] and prolonged or high-dose corticosteroid therapy can result in serious adverse events, including opportunistic infections such as central nervous system toxoplasmosis (Soeroso S: unpublished data). In northern Vietnam, mortality was 59% among 29 recipients of corticosteroids, as compared with 24% among 38 persons who did not receive corticosteroids ($P=0.004$) (Cao T, Thanh Liem N: personal communication). The possible value of other immunomodulators remains to be determined.

PREVENTION

Avian influenza A viruses are readily inactivated by a variety of chemical agents and physical conditions, including soaps, detergents, alcohols, and chlorination.[67,68] Guidelines for the prevention of infection with influenza A (H5N1) virus in various risk groups, including poultry workers, travelers, and health care workers, are available from the U.S. Centers for Disease Control and Prevention and the WHO.

ANTIVIRAL CHEMOPROPHYLAXIS

WHO guidelines for the use of antiviral agents for prophylaxis in persons who have been exposed to influenza A (H5N1) viruses in the current pandemic-alert period have been published.[61] Mathematical models of an emerging outbreak of influenza A (H5N1) in rural Asia predict that a strategy of mass, targeted antiviral chemoprophylaxis and social-distancing measures might extinguish or delay pandemic spread of the virus. The WHO has a stockpile of oseltamivir for this purpose and is working with partners for implementation of its distribution in the event of an outbreak.[69]

IMMUNIZATION

Safe and immunogenic inactivated H5 vaccines have been developed.[6] Reverse genetics permits the rapid generation of seed viruses with attenuated virulence, but the changing antigenicity of circulating strains of influenza A (H5N1) viruses calls for new candidate vaccines from different lineages[6] and the development of vaccines that elicit cross-clade immunogenicity. H5 hemagglutinin appears to be a weak human immunogen. For subvirion vaccines without adjuvants, persons who have not received a priming dose require two doses with a high hemagglutinin antigen content (Table 4 of the Supplementary Appendix). As compared with conventional subunit vaccines, certain oil-in-water adjuvant agents[6,70,71] or the use of whole-virus H5N1 vaccines[6,72,73] can substantially reduce the amount of vaccine antigen required to induce immune responses in persons who have not received a priming dose, and they can induce immune responses to antigenically drifted viruses. However, the specific adjuvant, formulation, dose, stability, and ratio with the antigen are important variables that require clinical testing for each candidate vaccine. Alum adjuvants have not consistently improved the responses to H5 vaccines,[6,73,74] whereas certain proprietary adjuvants (e.g., MF59 and AS03) appear to be highly effective and allow for considerable antigen-sparing and cross-reactive antibody responses.[6,70,71] These adjuvants have also been associated with increased rates of local and sometimes systemic reactogenicity.

The antibody levels required for protection against human influenza A (H5N1) illness are unclear. The durability of antibody responses is limited, but boosting with a homologous vaccine[70] or virus vaccine with viral antigen from another clade[75] appears to be effective in persons who have received two priming doses. Prepriming might allow single doses of a homologous vaccine to be

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sufficient for an antigenically drifted pandemic virus. However, decisions regarding the use of vaccine before a pandemic and stockpiling require complex risk–benefit and cost–benefit analyses that include effects on the seasonal capacity of vaccine production, because the timing and cause of the next influenza pandemic are unknown, and it is unclear whether immunization of large populations could have adverse consequences.

Initial studies in children and elderly persons suggest that antibody responses to subvirion vaccines at high doses (45 or 90 μg) are similar to those in young adults. Approximately 15 to 20% of older adults have some baseline neutralizing antibodies to H5N1 virus and may have a response to a single dose.[6] The mechanisms leading to these antibodies are uncertain. Other studies to date have shown that intradermal H5 vaccines at low doses are poorly immunogenic and may be associated with injection-site reactions.[6] Intranasal live attenuated H5 vaccines are highly effective in animal models,[76] but they show a variable ability to replicate in humans and to induce immune responses. Various investigational approaches, including conserved antigen vaccines, vectored H5 vaccines, and other adjuvants, are being explored.

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APPENDIX

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