Influenza

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Influenza is an acute respiratory illness, caused by influenza A, B, and C viruses, that occurs in local outbreaks or seasonal epidemics. Clinical illness follows a short incubation period and presentation ranges from asymptomatic to fulminant, depending on the characteristics of both the virus and the individual host. Influenza A viruses can also cause sporadic infections or spread worldwide in a pandemic when novel strains emerge in the human population from an animal host. New approaches to influenza prevention and treatment for management of both seasonal influenza epidemics and pandemics are desirable. In this Seminar, we discuss the clinical presentation, transmission, diagnosis, management, and prevention of seasonal influenza infection. We also review the animal–human interface of influenza, with a focus on current pandemic threats.

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Introduction

Influenza is an acute respiratory illness that has been recognised since the 16th century and spreads rapidly through communities in outbreaks.[1] Two forms of influenza occur globally: epidemic (seasonal or interpandemic) influenza caused by influenza A and B viruses, and sporadic pandemics caused by influenza A viruses. These epidemiological forms of influenza result from distinct mechanisms of antigenic variation in the surface glycoproteins of the virus, referred to as antigenic drift and antigenic shift. Antigenic drift is a continuous process that occurs in both influenza A and B viruses and results from the accumulation of point mutations in the viral haemagglutinin and neuraminidase genes. Antigenic drift is driven by antibody-mediated selective pressure and a high rate of viral mutations due to the absence of proofreading ability of the viral RNA-dependent RNA polymerase.[1,2] Antigenic drift permits the virus to escape immunity induced through previous exposure or vaccination, resulting in seasonal epidemics.[1] In temperate regions, influenza epidemics occur annually with a predictable seasonality, whereas in tropical regions they can occur all year round with unpredictable peaks.[3] Influenza epidemics spread rapidly with an average reproductive number of 1·28 and an attack rate of 10–20%, depending on age.[4–6] Influenza outbreaks are often first recognised in children presenting with febrile illness.[1] An increase in hospital admissions and respiratory or circulatory deaths are reported as the epidemic progresses. A typical influenza epidemic peaks within 2–3 weeks of onset and lasts 5–6 weeks.[1] Seasonal influenza accounts for thousands of deaths and hospital admissions annually in the European Union and the USA, with an even greater impact in developing countries.[7–11] Influenza epidemics in which H3N2 strains predominate are associated with the highest overall morbidity and mortality. The panel summarises the risk factors associated with severe illness, complications, or mortality due to influenza.

Antigenic shift is a sporadic event, restricted to influenza A viruses, and refers to the introduction into human beings of a novel virus strain to which a large proportion of the population does not have immunity.[1] If the novel influenza virus spreads efficiently and sustainably from person to person, it can cause a global pandemic. Four influenza pandemics have occurred in the past 100 years: H1N1 Spanish influenza in 1918, H2N2 Asian influenza in 1957, H3N2 Hong Kong influenza in 1968, and H1N1 swine influenza in 2009. Additionally, H1N1 viruses re-emerged in 1977 but did not cause a pandemic. During each pandemic, a novel influenza virus arose, either directly from an avian host (1918), via reassortment between an avian virus and a circulating human strain (1957 and 1968), or through influenza virus reassortment in pigs (2009), and spread through the human population, causing substantial morbidity and mortality, which was often associated with bacterial pneumonia.[1,2,20] The most severe pandemic occurred in 1918 and caused over 50 million deaths worldwide.[5,20] In the years following each pandemic, descendants of the pandemic strain established a new viral lineage in human beings and either replaced or co-circulated with previously circulating strains. Currently, the pandemic 2009 H1N1 (H1N1pdm09) influenza A virus is co-circulating with H3N2 and influenza B viruses.

Search strategy and selection criteria

We consulted a search specialist at the National Institutes of Health (NIH) library and searched the Cochrane Library and PubMed for articles published in the past 5 years (from Feb 1, 2011, to May 16, 2016) pertaining to influenza and each of the topics discussed in the Seminar. Search terms included "influenza", "influenza and systematic reviews", "influenza and diagnosis", "influenza and therapy", "influenza and prevention and control", "influenza and pandemic", "influenza and epidemiology", "influenza and clinical", "influenza and vaccines", "influenza and pandemic", "influenza and transmission", and "influenza and risk factors". The most relevant and recently published references were then selected to comply with the reference number limitations. Relevant textbook chapters and articles older than 5 years were included when indicated. Additional references that could be of interest to readers but are not directly cited in the article are included in the appendix.

See Online for appendix

Panel: Factors associated with increased morbidity and mortality from influenza[1,4,9,12–19]

Age
- Increased risk of death and hospital admission in individuals older than 65 years
- Increased risk of mortality from pandemic influenza in young adults (aged 20–40 years)
- Increased risk of hospital admission in children younger than 5 years (particularly children <2 years of age)

Pregnancy
- Highest risk of morbidity in third trimester
- High mortality observed in 1918 and 1957 pandemics
- Increased hospital admissions observed in the 2009 pandemic and during interpandemic influenza
- Poor fetal outcomes in pregnant women admitted to hospital for influenza

Immunocompromised state
- Mortality risk increased in individuals undergoing stem cell transplant, solid organ transplant, or chemotherapy
- Mortality risk increased in individuals with HIV who have a low CD4 cell count and are not on antiretrovirals
- Mortality risk increased in patients on other immune-modifying medications, but dependent on the degree of immune suppression

Medical comorbidity
- Presence of any medical comorbidity—including neuromuscular disease, cognitive dysfunction, pulmonary disease, cardiovascular disease, renal disease, liver disease, diabetes, heavy alcohol use, and obesity—associated with increased mortality risk
- Individual comorbidities associated with variable risk of admission to hospital and mortality

Genetic susceptibility
- Increased risk of hospital admission for influenza in individuals carrying certain alleles of interferon-induced transmembrane protein 3 (*IFITM3*);[19] mechanism is under investigation; *IFITM3* restricts cellular entry of influenza viruses and is an important interferon-stimulated gene

The virus

Influenza viruses belong to the Orthomyxoviridae family and are divided into three types (A, B, and C).[1,21,22] Influenza A and B viruses cause seasonal epidemics, whereas influenza C viruses generally cause mild disease. Influenza A viruses are further classified into subtypes on the basis of the antigenic properties of their two surface glycoproteins, haemagglutinin and neuraminidase. 16 haemagglutinin and nine neuraminidase subtypes of influenza A viruses have been isolated from birds (H1 to H16 and N1 to N9), and RNA of an additional two haemagglutinin and neuraminidase subtypes has been identified in bats (H17 and H18, and N10 and N11).[23] A similar animal reservoir does not exist for influenza B viruses but two antigenically distinct lineages of influenza B viruses—Victoria and Yamagata—co-circulate in human beings.[1]

Influenza viruses are enveloped particles that contain a single-stranded, segmented RNA genome.[1,22] Influenza A and B viruses possess eight gene segments, which encode at least 17 proteins. The haemagglutinin protein mediates binding of influenza virus to its receptors, sialyloligosaccharides on the host cell.[1,22,24] Human influenza viruses preferentially bind to α2,6-linked sialyloligosaccharide receptors, which predominate in the human upper respiratory tract, whereas avian influenza viruses bind to α2,3-linked sialyloligosaccharide receptors, which are more prevalent in the lower respiratory tract.[25] The neuraminidase protein facilitates viral particle release by cleaving sialyloligosaccharide residues from the host cell surface.[1,22] The viral haemagglutinin, neuraminidase, and matrix 2 proteins are targets of the protective antibody response, and the nucleoprotein and matrix 1 proteins are targets of the cellular immune response.[26]

Transmission

The epidemiological success of influenza viruses lies in their ability to spread efficiently from person to person. Three mechanisms of influenza transmission have been identified—aerosol, droplet, and contact transmission—and the relative importance of each mechanism is a matter of debate.[1,27] When an infected individual sneezes or coughs, they expel infectious particles ranging from 0·1 μm to 100 μm in diameter.[28] Fine particles (aerosols) and droplet nuclei, generated from the rapid desiccation of larger droplets, have a diameter less than 5 μm and are able to remain airborne for minutes to hours, but are vulnerable to changes in temperature and humidity.[29,30] They can be inhaled and deposited in the upper or lower respiratory tract.[1,27] Larger droplets are deposited in the upper respiratory tract or settle quickly in the environment, generally within 2–3 m of the infected individual. Contact transmission from fomites to mucosal surfaces can occur.[27,31] The virus remains infectious for a short time on the hands but can remain infectious on non-porous surfaces in the environment for up to 48 h. Previously, most influenza transmission events were thought to occur via large droplets. Below, we describe data supporting a role for aerosol transmission of influenza viruses and the implications for infection control policy.

Ferrets experimentally infected by aerosol inoculation have influenza infections that more closely resemble naturally acquired human illness than ferrets inoculated intranasally.[32] Experimental infections of human volunteers also support a role for aerosol transmission of influenza, and the minimum infectious dose via aerosol is lower than when the virus is administered through intranasal drops (0·6–3·0 median tissue culture infectious doses [$TCID_{50}$] for aerosol transmission compared with 127–320 $TCID_{50}$ for intranasal inoculation).[33] Influenza viruses have been detected in the air from patients' rooms, urgent care centres, and emergency rooms, and epidemiological observations point to a substantial contribution of aerosol transmission in outbreak settings.[1,29,30,34,35] Moser and colleagues[36] described the spread of influenza aboard an

airliner where the ventilation system was shut off for several hours; 38 (72%) of the 53 passengers developed influenza. Aerosol transmission has also been suggested to contribute to the spread of influenza in a tuberculosis ward in California, USA, a medical ward in Hong Kong, a skilled nursing facility in Wisconsin, USA, and in household contacts in China.[31,37–39]

WHO and the US Centers for Disease Control and Prevention (CDC) recommend the use of a surgical mask when caring for a patient with influenza; respirators (N95 or powered air purifying respirators) are only recommended during aerosol-generating procedures such as bronchoscopy or intubation.[40,41] Surgical masks and respirators appear to provide health-care workers with a similar degree of protection from transmission of influenza viruses.[28,42] Data suggest that the use of surgical masks can prevent most influenza transmission events in health-care settings with appropriate air exchange, good hand hygiene practices, and immunity to seasonal influenza through previous exposure or vaccination. However, aerosols can play an important role in influenza transmission. Thus, respirator use is recommended during aerosol-generating procedures and might also be prudent for all patient care activities during an influenza pandemic when population immunity is low.

Clinical presentation

The presentation of seasonal influenza ranges from an asymptomatic infection to a fulminant illness, depending on the characteristics of both the host and virus.[1,12,21,43] Symptoms appear suddenly after an incubation period of 1–2 days and are characterised by various systemic features, including fever, chills, headache, myalgia, malaise, and anorexia, accompanied by respiratory symptoms, including non-productive cough, nasal discharge, and sore throat.[1,12,21,43] Ocular symptoms can also be present and include photophobia, conjunctivitis, lacrimation, and pain with eye movement.[43]

When present, fever is the most important physical finding and temperatures can be as high as 41°C in the first 24 h of illness.[1,12,21,43] Physical examination can reveal a toxic appearance with prominent flushing of the face and hyperaemic mucous membranes.[43] A clear nasal discharge might be present and eyes might be injected or watery. Small cervical lymph nodes might be palpable and tender. About 25% of cases have diffuse rhonchi or rales upon auscultation of the lungs. Fever and associated systemic symptoms typically last for 3 days but can persist for up to 8 days. Cough and malaise can persist for up to 2 weeks after resolution of fever.

The presentation of influenza in children can differ from that in adults.[21,43] Children have higher maximum temperatures than do adults, and infants can present with an undifferentiated fever or febrile seizures. Laryngotracheobronchitis (croup), bronchiolitis, and bronchitis can occur. Children report severe myalgia in the calf muscles, and myositis is a more frequent complication than in adults.[1,21,43] Gastrointestinal symptoms are seen with higher frequency in children than in adults.[1,21,43]

Viral shedding typically begins during the incubation period, peaking in the first 1–2 days of clinical illness, and decreasing to undetectable amounts after a week, correlating well with the severity of clinical symptoms.[44] Children and immunocompromised patients shed virus for a longer duration than do healthy adults.[13,45] There is a paucity of data regarding the shedding of influenza virus in asymptomatic infections.[46]

Pulmonary complications

Primary influenza-associated pneumonia was first documented during the 1957 pandemic, although it was suspected during the 1918 pandemic.[1,43,47] Patients present with typical influenza symptoms, followed by rapid respiratory decompensation. Chest imaging reveals diffuse bilateral infiltrates, and sputum cultures are negative for bacteria. Mortality is high and autopsy reveals necrotising bronchitis, hyaline membranes, intra-alveolar haemorrhage and oedema, and interstitial inflammation.

Bacterial pneumonia as a complication of influenza infection was first documented during the 1918 pandemic as a biphasic illness in which typical influenza symptoms occur and then resolve, followed 4–14 days later with a recurrence of fever associated with dyspnoea, productive cough, and consolidation on chest imaging.[43,47] The most common organisms isolated from sputum are *Streptococcus pneumoniae*, *Staphylococcus aureus* (including community-acquired meticillin-resistant *S aureus*), *Haemophilus influenzae*, other *Streptococcus* species, and other Gram-negative rods.[12,43,47] A large proportion of fatalities during the 2009 pandemic was associated with bacterial pneumonia.[48,49] Combinations of primary influenza-associated pneumonia and secondary bacterial pneumonia can occur.

Influenza infection is associated with bronchiolitis and croup.[12,21,43] The infection can lead to exacerbations of underlying chronic lung disease such as asthma, chronic obstructive pulmonary disease, and chronic bronchitis, and a decline in lung function in individuals with cystic fibrosis.

Non-pulmonary complications

In addition to pulmonary complications, several effects on other organ systems can be seen in influenza.[1,12,21,43,47,50] Myositis and rhabdomyolysis occur rarely, with varying severity. Difficulties with ambulation and renal failure can occur and can persist for 4–6 weeks. Cardiac complications of influenza include myocarditis, pericarditis, and exacerbation of underlying cardiac disease. Influenza has also been associated with neurological manifestations, including Reyes syndrome, encephalomyelitis, transverse myelitis, Guillain-Barré syndrome, aseptic meningitis, and encephalitis. Reyes syndrome is characterised by acute encephalopathy without evidence of inflammation on analysis of cerebrospinal fluid, associated with liver

	Sensitivity	Turnaround time	Advantages	Disadvantages
Viral culture	Close to 100%	3–10 days	High sensitivity and specificity; virus available for characterisation (recovery of new and divergent strains); ability to recover other viruses	Poor specimen quality might affect yield; results not available in time to inform clinical decision making; time and labour intensive; specialised laboratory facilities required
Rapid viral culture*	70–90%	1–3 days	Faster than traditional viral culture; less expertise needed than for traditional cell culture	Less sensitive than traditional viral culture; might miss divergent influenza viruses; specialised laboratory facilities required
Rapid antigen detection: direct fluorescent antibody	70–90%	1–4 h	Rapid turnaround; can identify additional pathogens (different staining methods); can assess sample quality	Sensitivity and specificity dependent on expertise of technician; specialised equipment required; virus is not available for characterisation of antigenicity
Rapid antigen detection: immunochromatogenic assay	59–93%	<30 min	No specialised equipment or technical skill required; specialised specimen transport not required; rapid results	Least sensitive method; virus is not available for characterisation of antigenicity
RT-PCR	Close to 100%	1–8 h	High sensitivity and specificity; specimen quality and handling have less impact on sensitivity; typing, subtyping, and sequencing possible; can be combined with multiplex technology	Expensive; specialised equipment and trained personnel required; potential for cross-contamination; might miss divergent strains (dependent on primers)

*This technique is a modification of conventional viral culture, where the clinical specimen is inoculated onto a cell monolayer and centrifuged before incubation. Specimens are then stained and examined by immunofluorescence.

Table 1: **Comparison of methods for diagnostic testing of influenza**[1,21,40,51,59]

function abnormalities and elevated serum ammonia concentrations. This syndrome occurred mostly in children receiving aspirin and is now a rare event because aspirin use in children has declined substantially.

Diagnosis

Clinical diagnosis of influenza is difficult because symptoms range in severity and overlap with those caused by other respiratory viruses.[51] The sensitivity and specificity of clinical diagnosis are influenced by the case definition used, the characteristics of the host, and the prevalence of influenza in the community.[52–56] In healthy adults, the sensitivity of clinical diagnosis ranges from 29% to 80%.[52–55] Clinicians are likely to appropriately diagnose influenza infection when fever and cough are part of the case definition, when influenza rates are high in the community, and when patients are severely ill or are at an increased risk of developing complications. Laboratory tests are available to aid in the diagnosis and can be used to guide treatment decisions, avoid inappropriate use of antibiotics, and provide information for influenza surveillance.[1,40,57–60] Physicians should be aware of influenza rates in the community and use laboratory tests when the results will influence clinical management.

Influenza testing should be done early in the course of the illness, when viral shedding is at its peak.[51,59] The preferred samples include nasopharyngeal swabs, nasal washes, and nasopharyngeal aspirates. Lower respiratory tract samples such as bronchoalveolar lavage and endotracheal aspirates can also be tested and might be more sensitive in individuals with influenza-associated pneumonia.[61] Recommended diagnostic methods include viral culture, antigen detection, and nucleic acid testing.[1,40,57–60] The sensitivities and advantages of these modalities are summarised in table 1. Serological testing for influenza to aid in clinical decision making is not recommended, but it can be useful in clinical studies and outbreak investigations.[40,51]

Licensed therapeutics

Four classes of antiviral drugs are approved for the treatment of influenza in several countries: adamantanes, neuraminidase inhibitors, membrane fusion inhibitors, and RNA-dependent RNA polymerase inhibitors.[62] Of these, only the adamantane derivatives and neuraminidase inhibitors are licensed for use in the European Union and the USA.[40] The adamantane derivatives include two oral agents, amantadine and rimantadine, which inhibit the matrix 2 ion channel of influenza A, but not B, viruses.[1] Point mutations in the membrane spanning region of the matrix 2 protein confer resistance to both amantadine and rimantadine while preserving viral fitness.[63] All currently circulating seasonal influenza viruses are resistant to the adamantane derivatives and so the use of these agents is not recommended.[10,40]

Neuraminidase inhibitors inhibit the function of the influenza virus neuraminidase.[1] During the 2015–16 influenza season, oral oseltamivir and inhaled zanamivir were recommended for use in the European Union and the USA.[10,40] Intravenous peramivir is also recommended for use in the USA, and intravenous zanamivir is available for severely ill patients with suspected

	Route	Treatment dose	Prophylactic dose	Special population considerations
Oseltamivir (Tamiflu; Roche)	Oral	Adults: 75 mg twice daily for 5 days Children (2 weeks and older): weight-based dosing twice a day for 5 days	Adults: 75 mg once daily for 7–10 days Children (3 months and older): weight-based dosing once a day for 7–10 days	Available in the USA and European Union; dose adjusted in renal failure; drug of choice in pregnancy; drug of choice in patients with severe infection or patients admitted to hospital; side-effects include nausea or vomiting, rare and serious skin reactions, and neuropsychiatric effects (observed in post-marketing studies in Japan)
Zanamivir (Relenza; GlaxoSmithKline)	Inhaled	7 years and older: 10 mg twice daily for 5 days	5 years and older: 10 mg once daily for 7–10 days	Available in the USA and European Union (except for Cyprus); contraindicated in patients with underlying lung disease; contraindicated in intubated patients (ventilator blockage)
Peramivir (Rapivab; BioCryst Pharmaceuticals)	Intravenous	18 years and older: 600 mg in a single dose	NA	Available in the USA; dose adjusted in renal failure; given for at least 5 days if used to treat patients in hospital

NA=not applicable.

Table 2: Treatment options for influenza infections in the USA and Europe[1,10,40,64]

oseltamivir-resistant influenza on a compassionate basis by emergency investigational drug request.[40] Table 2 describes recommended options for antivirals in the European Union and the USA. Prophylaxis is recommended in unvaccinated individuals at high risk of developing complications after exposure to influenza virus (eg, after close contact with infected individuals) and for control of outbreaks in an institutional setting (in an institutional outbreak, the antiviral agent should be continued for at least 2 weeks or 7 days after the last documented infection occurs).[40] Treatment of influenza infection is indicated for patients admitted to hospital with suspected or confirmed influenza and individuals at high risk of developing influenza-related complications. Treatment can also be considered for uncomplicated influenza infections in low-risk individuals who present within 48 h of symptom onset.[40]

Resistance to neuraminidase inhibitors can occur through multiple mechanisms.[65] Some resistance-conferring mutations change the catalytic framework of the neuraminidase molecule or lead to internal deletions in the neuraminidase so that the drug cannot bind, whereas others alter the haemagglutinin so that neuraminidase activity is not required to release the virus from the infected cell.[66,67] These mutations confer variable changes in viral fitness and could yield resistance to one or more of the available neuraminidase inhibitors. During the 2007–08 influenza season, substantial resistance to oseltamivir emerged in influenza A H1N1 viruses through a histidine to tyrosine substitution (H275Y) in the neuraminidase protein.[66] Since the emergence of H1N1pdm09 influenza A viruses and their establishment as circulating epidemic strains, resistance to neuraminidase inhibitors has been uncommon.[68] As of March, 2016, all circulating influenza A H3N2 and influenza B isolates in the USA were susceptible to the licensed neuraminidase inhibitors and only 5% of influenza A H1N1pdm09 isolates were resistant to oseltamivir and peramivir.[40] Rates of oseltamivir resistance were lower than 5% in the European Union, with resistance observed in less than 1% of influenza A H1N1pdm09 isolates.[10]

Controversies in therapeutics

Although neuraminidase inhibitors are widely used, the effectiveness of these agents has been the subject of much debate. All three licensed neuraminidase inhibitors are most effective when given early in infection, preferably within 48 h of onset of illness.[40] In randomised controlled trials of uncomplicated influenza in healthy outpatients, neuraminidase inhibitors shortened the duration of clinical symptoms by less than 1 day.[69–72]

Data on the effectiveness of neuraminidase inhibitors in the prevention of influenza-related complications are variable. A 2014 Cochrane review[71] found no decrease in the risk of hospital admissions (risk difference [RD] 0·15%; 95% CI –0·78 to 0·91) or serious complications with oseltamivir treatment (0·07%; –0·78 to 0·44). Data analysis was done in an intention-to-treat (ITT) group without accounting for the results of influenza testing. A subsequent meta-analysis, by Dobson and colleagues,[69] divided individuals into an ITT group and an ITT infected (ITTI) group, in which influenza infection was confirmed by testing. This study estimated a 44% risk reduction (relative risk 0·56 [95% CI 0·42–0·75]; p=0·0001) in lower respiratory tract complications and a 63% risk reduction (0·37 [0·17–0·81]; p=0·013) in hospital stay for the ITTI group that received oseltamivir.[69]

The 2014 Cochrane review[71] found that prophylaxis with oseltamivir or zanamivir had a modest effect on prevention of symptomatic influenza illness in individuals (RD 3·05% [95% CI 1·83–3·88] for oseltamivir; 1·98% [0·98–2·54] for zanamivir) and a slightly better effect in households (13·6% [9·52–15·47] for oseltamivir; 14·8% [12·18–16·55] for zanamivir).[70,72] Additional studies, showing that prophylaxis can reduce household transmission of influenza, lend support to the use of these agents in a pandemic setting, although large, community-based studies have not been done.[73,74]

	Mechanism of action	Route of administration	Licence or investigational status
Licensed antiviral agents			
Laninamivir	Neuraminidase inhibitor	Inhaled	Licensed in Japan for seasonal influenza
Arbidol	Interaction with haemagglutinin to prevent membrane fusion	Oral	Licensed in Russia and China for seasonal influenza
Favipiravir (T-705)	Inhibition of viral RNA-dependent RNA polymerase	Oral	Licensed in Japan for pandemic influenza
Investigational antiviral agents			
Monoclonal antibodies (anti-haemagglutinin head, anti-haemagglutinin stem, anti-M2e)	Neutralisation of virus; antibody effector function; stimulation of immune response	Intravenous	Preclinical; clinical trials
Nitazoxanide	Interferon induction; inhibitor of haemagglutinin maturation	Oral	Approved for parasitic infections; preclinical; clinical trials
DAS181	Sialidase	Inhaled	Preclinical; clinical trials
VX-787	Inhibition of polymerase basic protein 2 (PB2)	Oral	Preclinical; clinical trials
AVI-7100	Small interfering RNA construct	Intravenous	Preclinical; clinical trials

Table 3: **Antivirals licensed for influenza treatment outside Europe and USA, and investigational antiviral agents**[81,82,87–92]

Many countries recommend oral oseltamivir for treatment of complicated influenza, primarily on the basis of observational data, which suggest a mortality benefit in patients treated with oseltamivir.[10,49,41,75–77] A meta-analysis[76] of individual patient data for 29 234 influenza-infected individuals admitted to hospital between January, 2009, and March, 2011, reported a lower risk of mortality in individuals treated with a neuraminidase inhibitor than in untreated individuals (adjusted odds ratio 0·81; 95% CI 0·70–0·95; p=0·0024). Earlier treatment was associated with better outcomes than later initiation of therapy. In other observational studies, treatment delayed by more than 48 h after symptom onset, increased durations of therapy, and higher doses of oseltamivir have been used in severely ill patients, but efficacy data are not available.[40,76,78–80]

Taken together, these data support the use of neuraminidase inhibitors for prophylaxis against influenza infections in both individual patients and outbreak settings. When used for treatment, neuraminidase inhibitors provide a slight benefit in healthy adults, shortening the duration of illness by approximately 1 day. Additional data are needed to better assess the use of neuraminidase inhibitors in high-risk or severely ill patients, but in the setting of limited treatment options and acceptable side-effect profiles these agents are widely used.

Research on therapeutics

Controversial efficacy data and the potential for drug resistance make adjuncts to current treatment, as well as the development of novel therapeutics, important areas of research. Combinations of antiviral agents have an additive treatment benefit in preclinical studies.[81] A triple combination of amantadine, ribavirin, and oseltamivir showed synergistic activity, in vitro, against both adamantane-sensitive and adamantane-resistant viruses. In a retrospective observational study of critically ill adults infected with H1N1pdm09 virus, this regimen did not show a significant decrease in 14-day mortality (17% *vs* 35%; p=0.08) and 90-day mortality (46% *vs* 59%; p=0·23) compared with oseltamivir treatment alone.[81] Several low-cost, widely available immunomodulatory agents, including statins and macrolides, have been used in animal models and human beings as adjuncts to neuraminidase inhibitors for influenza treatment, with variable results.[81–84] Corticosteroids have also been used as adjuncts to antivirals, particularly in critically ill patients.[81,85,86] Data are scarce, but observational studies from the 2009 influenza pandemic suggest the frequency of secondary infections, duration of intensive care unit stays, and mortality are all increased in patients treated with corticosteroids. Several antiviral agents are licensed for influenza treatment outside the European Union and USA (table 3). Additionally, various promising antiviral drugs with novel mechanisms of anti-influenza activity have shown efficacy in animal models or initial clinical studies (table 3).[81,82,87–92]

Vaccination

The most effective method for prevention and control of influenza infection is vaccination.[1,22,93] Licensed seasonal vaccines are updated annually and WHO makes recommendations on the composition of the next season's influenza vaccines on the basis of surveillance, laboratory, and clinical observations.[93] This process occurs twice a year, in February for the northern hemisphere and in September for the southern hemisphere. Tropical countries follow one of these recommendations. Procuring the influenza vaccine can be difficult for people planning to travel to the opposite hemisphere during the local influenza season.

Table 4 summarises the vaccines that are currently recommended for seasonal use in the USA. Vaccine availability varies by country in the European Union.

Three classes of licensed influenza vaccines are available: inactivated virus, live attenuated virus, and recombinant haemagglutinin vaccines.[40,93,94] Available

	Quadrivalent vs trivalent*	Route	Approved age group	Comments
Inactivated	Quadrivalent or trivalent	Intramuscular	≥6 months	Contains 15 µg of each haemagglutinin
Inactivated: intradermal	Quadrivalent	Intradermal	18–64 years	Contains 9 µg of each haemagglutinin
Inactivated: derived from cell culture	Trivalent	Intramuscular	≥18 years	Contains 15 µg of each haemagglutinin; contains egg protein; manufacturing does not rely on eggs
Inactivated: high dose	Trivalent	Intramuscular	≥65 years	Contains 60 µg of each haemagglutinin
Live attenuated†	Quadrivalent	Intranasal	2–49 years	Cold adapted; uses a master donor virus plus the haemagglutinin and neuraminidase of the circulating viruses; generates a broader immune response (T-cell, mucosal); not approved for use in immunocompromised patients or pregnant women
Recombinant	Trivalent	Intramuscular	≥18 years	Made with recombinant DNA technology to produce full-length haemagglutinin; shorter manufacturing time than for egg-derived or cell-culture-derived vaccines; can be used in individuals with egg allergy

*Trivalent vaccines contain antigens from the circulating H1N1 and H3N2 influenza A viruses and the dominant influenza B virus circulating at the time of vaccine strain selection. Quadrivalent vaccines contain antigens from the circulating H1N1 and H3N2 influenza A viruses and both lineages of influenza B. †Live attenuated vaccine not recommended by the US Advisory Committee on Immunization Practices for the 2016–17 season; this table represents the 2015–16 influenza season.

Table 4: **Types of influenza vaccine licensed for use in the USA 2015–16 influenza season**[40,93-97]

vaccines contain antigens from the influenza A H1N1 and H3N2 subtypes, along with the dominant circulating lineage of influenza B (trivalent vaccines) or both lineages of influenza B (quadrivalent vaccines). Previously, the use of live attenuated influenza vaccines was recommended in young children in the USA on the basis of favourable clinical trial results and observational data in this age range.[95,98] In 2016, Gaglani and colleagues[99] analysed data from the US Flu Vaccine Effectiveness Network for the 2013–14 influenza season in fully vaccinated children aged 2–17 years, and showed that for influenza A H1N1pdm09 the live attenuated vaccine provided inferior protection (effectiveness 17%; 95% CI −39 to 51) to that of the inactivated vaccine (60%; 36 to 74). This finding and similar effectiveness data during the 2015–16 influenza season led the US Advisory Committee on Immunization Practices to recommend that live attenuated vaccines are not used in the 2016–17 influenza season.[40] By contrast, European data have continued to show protection with live attenuated vaccines in children, although effectiveness against influenza A H1N1pdm09 is lower than against influenza B.[100] The live attenuated vaccine continues to be recommended in many European countries, and further investigation is needed to optimise vaccination strategies.

The ability of vaccines to protect a target population is assessed in vaccine efficacy and effectiveness studies.[101] Vaccine efficacy refers to the specific reduction in the rates of laboratory-confirmed influenza and is assessed in randomised controlled trials. Vaccine effectiveness is determined by observational data and assesses other endpoints besides laboratory-confirmed influenza, including influenza-like illness, medically attended respiratory illness, the burden of missed work, and hospital admissions, during the influenza season.[40] Vaccine effectiveness studies now use a test-negative design in which individuals who present to health-care settings with an influenza-like illness and test positive for influenza are assessed for vaccination status.[101] These studies have shown that influenza vaccination is most effective when the vaccine strain matches the circulating epidemic strain.[93] Effectiveness rates of 50–60% are reported for well matched influenza vaccines in healthy adults and children.[40,102,103] Vaccine benefit is greatest among high-risk groups such as individuals older than 65 years (especially those with comorbidities), immunocompromised patients, and young children.[104–107] In the 2013–14 influenza season, the CDC estimated that influenza vaccination led to 90 068 (95% CI 51 231–144 571) fewer overall hospital admissions than if no vaccination had taken place.[107] When seasonal influenza viruses undergo antigenic drift after a vaccine has been distributed, a marked decrease in vaccine effectiveness occurs.[40] Vaccine effectiveness may also be influenced by previous vaccination. Some studies have suggested that annual vaccination could result in decreased vaccine effectiveness,[108,109] but additional data are needed to investigate this issue and its implications for vaccine policy.

In Europe, recommendations for routine annual influenza vaccination and rates of vaccination coverage vary by country.[10] Most countries recommend influenza vaccination in individuals at high risk of developing complications, including elderly people, pregnant women, individuals with medical comorbidities, residents of long-term care facilities, and health-care workers. In the USA, annual influenza vaccination is recommended for all individuals aged 6 months or older and is particularly emphasised for individuals at high risk of developing complications of influenza infection, and for health-care workers.[94] However, the CDC estimated that in the 2014–15 influenza season only 47% of children and adults were vaccinated, with the lowest rates (33%) seen in healthy adults aged 18–49 years.[40] Higher rates were seen in adults older than 65 years

(67%), adults aged 18–64 years with high-risk conditions (48%), pregnant women (50%), and children aged 6 months to 17 years (59%). 77% of health-care personnel were vaccinated, but rates as low as 64% were reported among health-care personnel in long-term care facilities.[96]

Only 40–50% of pregnant women in the USA were vaccinated during the 2014–15 influenza season.[110] Maternal vaccination is the primary mechanism to protect young infants because vaccines are not licensed for infants younger than 6 months.[111] However, pregnant women frequently defer vaccination because of concerns regarding the safety of the influenza vaccine during pregnancy. Several reviews have shown no increase in adverse maternal or fetal effects after administration of inactivated seasonal influenza or H1N1pdm09 influenza vaccines.[112–114] Pregnant women should therefore be informed of the benefits of vaccination for themselves and their infants, and should be reassured of the safety of inactivated influenza vaccines.

Antigenic drift of seasonal influenza viruses and the emergence of novel influenza viruses by antigenic shift, combined with the time required to develop an influenza vaccine, make new vaccine approaches an important component of influenza research. New approaches such as DNA-based vaccines, viral vectors, virus-like particles, cell-culture techniques, recombinant DNA, novel live attenuated vaccines, and adjuvants are being studied to improve vaccine development and immunogenicity.[115] Oil-in-water adjuvants, such as MF59 and AS03, improve immune responses to inactivated influenza vaccines, particularly in young children (aged 6 months and older) and people older than 60 years.[116,117] Several such vaccines have been licensed in Europe for seasonal influenza and in the USA for individuals at high risk of exposure in the event of an H5N1 pandemic. Generally, oil-in-water adjuvants are well tolerated with a side-effect profile similar to that of non-adjuvanted inactivated vaccines.[117] However, results of retrospective studies[118,119] in Europe suggested an association between Pandemrix (GlaxoSmithKline), an AS03-adjuvanted H1N1pdm09 vaccine, and the subsequent development of narcolepsy in vaccinated individuals younger than 21 years. The role of the AS03 adjuvant in this association is unclear, and other factors—including a particular HLA type—might have a role.[117] A universal influenza vaccine that is broadly cross-protective could replace annual seasonal influenza vaccination and provide protection following the emergence of a novel influenza virus.[93,120,121] Vaccines based on various viral targets, such as the haemagglutinin stem, matrix 2 protein, and consensus sequences of the haemagglutinin head, are in development.[93,115]

Ecology

Influenza A viruses have been isolated from several species besides human beings, including birds, pigs, dogs, cats, horses, and marine mammals.[122] Waterfowl and shorebirds are the natural reservoir of influenza A viruses and the source of all strains that infect domestic avian species and mammals.[2]

In waterfowl, influenza infection causes localised respiratory or gastrointestinal infection without overt clinical manifestations.[2] Influenza A viruses replicate predominantly in the intestinal tract of waterfowl and are spread through faecal contamination of water.[122] Transmission of influenza A viruses from wild birds to domestic poultry occurs on farms or in rice paddies along migratory bird flyways, in backyard poultry farms, and in live bird markets where multiple avian species are in close proximity. Domestic poultry are not natural hosts for influenza viruses and have a range of clinical manifestations, from asymptomatic infection or decreased egg production to severe multisystem disease and rapid death.[2] Severe disease in poultry is linked to the properties of the viral haemagglutinin.[24,122] Low pathogenicity avian influenza viruses have haemagglutinin molecules that can only be cleaved by proteases in the gastrointestinal tract of waterfowl, whereas highly pathogenic avian influenza viruses of the H5 and H7 subtypes have molecular motifs that allow the haemagglutinin of these viruses to be cleaved by proteases outside the gastrointestinal tract, resulting in disseminated multisystem infection. Both high pathogenicity and low pathogenicity avian influenza viruses can cause severe disease in human beings.[122]

Interspecies transmission of influenza A viruses is a complicated process that is restricted by both viral and host factors. The receptor specificity of the haemagglutinin protein binding to sialyloligosaccharides on the host cell is an important determinant of the host range of influenza A viruses.[2] Aminoacid changes in the haemagglutinin receptor binding domain can facilitate interspecies transmission. The viral polymerase genes have also been implicated in host-range restriction and virulence.[24] The replication efficiency of an influenza virus corresponds to its virulence.[123] Certain aminoacid substitutions in the polymerase proteins can enhance the pathogenicity of influenza viruses.[124] The best-described aminoacid change is a glutamic acid to lysine substitution at position 627 in polymerase basic protein 2 (PB2). Most avian influenza viruses have a glutamic acid at this position, whereas most human viruses have a lysine.[125] Several other viral and host factors have been implicated in interspecies transmission of influenza A viruses, and research is ongoing to further delineate the mechanisms involved in this complex process.

Emerging pandemic threats

H5N1

In 1997, a 3-year-old boy in Hong Kong developed a respiratory illness and died of acute respiratory distress syndrome.[126] An influenza virus isolated from this patient was composed entirely of avian influenza virus genes and constituted the first detection of H5N1 avian influenza A infection in human beings. According to WHO, 856 cases

and 452 deaths have occurred from avian influenza A H5N1 infection in 16 countries as of Oct 3, 2016, with the largest number of cases in Indonesia, Vietnam, and Egypt.[41,120] Human cases of avian influenza A H5N1 infection are typically associated with exposure to infected poultry while butchering, defeathering, and preparing sick birds for consumption, or associated with live bird markets.[127] Isolated person-to-person transmission of H5N1 in close contacts has been reported, but sustained transmission has not occurred.[20] H5N1 infection in human beings begins after an incubation period of 2–5 days, with rapid progression of the disease.[128] Respiratory failure and multi-organ dysfunction are common, and case-fatality rates reach as high as 60%.[129] Over 90% of reported cases are in individuals younger than 40 years of age. The prevalence of asymptomatic or mild infection is unknown, but seroprevalence studies have documented H5N1-specific antibodies in a minority of individuals with high-risk exposures.[128,130] Extensive adamantane resistance has been documented in H5N1 influenza strains, but most strains remain susceptible to neuraminidase inhibitors.[129] Early administration of oseltamivir in H5N1 infection is recommended, but data to support this action are mostly observational and case-fatality rates are high despite oseltamivir use. Avian H5N1, H5N2, and H5N8 viruses have been detected in birds in the USA and avian H5N8 viruses have been detected in birds in Europe, but human cases have not been reported despite documented exposure.[40]

H7N9

Before 2013, with the exception of one fatal case, sporadic cases of infection with H7 subtype viruses (H7N7, H7N3, and H7N2) were associated with mild illness.[131] In 2013, a novel H7N9 virus emerged in China and has reappeared annually, resulting in 798 reported cases and 320 deaths as of Oct 3, 2016.[41,132] Most human cases of avian influenza A H7N9 are associated with exposure to poultry, although rare cases of person-to-person transmission have been reported.[131,133] The H7N9 virus often circulates undetected in poultry, since it is a low pathogenicity avian influenza virus.[120] Thus, infections in human beings occur without any warning unless influenza surveillance is done among local poultry. Human H7N9 infection causes a severe respiratory illness.[134] As with seasonal influenza, infection is most severe in elderly people and in individuals with comorbidities. A faster overall increase has been observed in the number of human cases of H7N9 compared with H5N1 virus infections.[41,134] The H7N9 virus shows extensive resistance to the adamantanes, but most strains are susceptible to oseltamivir.[135] Although efficacy data are scarce, oseltamivir is recommended for treatment of H7N9 infection.

Other influenza subtypes

In addition to the subtypes discussed above, human infections with other avian and swine influenza A viruses have been documented, including H9N2, H10N8, H10N7, and H6N1.[2,120,131] Most infections are characterised by mild respiratory illness or conjunctivitis, but H10N8 infection was associated with cases of severe pneumonia in China.[120] H9N2 viruses are of particular interest because they are the source of the internal protein genes of the H7N9, H6N1, and H10N8 viruses in China. H9N2 viruses in Asian poultry have also shown receptor specificity similar to that of human influenza viruses, although to date only 15 human infections with H9N2 influenza A virus have been documented, with no evidence of person-to-person transmission.[131,136,137]

Influenza viruses cause seasonal epidemics in pigs.[138] Circulating viruses in the US swine population include H1N1, H3N2, and H1N2 subtypes. Because both α2,6-linked and α2,3-linked sialyloligosaccharide receptors are found in the porcine respiratory tract, both avian and human influenza A viruses can cause illness in pigs.[122] Seasonal human influenza viruses are commonly transmitted and established in pigs.[138] These viruses have the capacity to reassort within the swine population, and such events are thought to have generated the H1N1pdm09 influenza A virus. Sporadic transmission of swine influenza A viruses to human beings can also occur.[131] These viruses are called variant viruses and are denoted by adding the letter "v" to the end of the virus subtype. Human infections with H1N1v, H3N2v, and H1N2v viruses have occurred in the USA.[40] H3N2v infections account for the majority of reported cases and have caused 391 illnesses since they were first detected in August, 2011. Cases are generally reported in individuals who have had close contact with pigs either through occupational exposure or at agricultural fairs.[122] Some degree of person-to-person transmission has been documented.[139] Most influenza variant virus infections have occurred in children, presumably because the prevalence of cross-reactive antibodies increases with age.[40] The illness is generally mild and is similar to uncomplicated seasonal influenza.

In summary, animal influenza viruses can directly infect human beings and, as with human influenza viruses, the resulting clinical illness can range from mild to severe. A history of exposure to sick birds or travel to countries where cases of avian influenza are occurring might raise suspicion of a novel influenza virus infection, but a definitive diagnosis requires laboratory tests that are usually only available through public health reference laboratories. Identification of such infections is important for determining the source of the virus, evidence of person-to-person spread, and assessment of pandemic potential.

Contributors

CP and KS contributed equally to the design, writing, and review of this Seminar.

Declaration of interests

KS has a patent issued for pandemic influenza vaccines, and was the principal investigator on a cooperative research and development agreement with MedImmune to generate and evaluate pandemic

influenza vaccines. Neither KS nor her laboratory received financial support from MedImmune. CP declares no competing interests.

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