Seminar

Influenza

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Influenza is the most frequent cause of acute respiratory illness requiring medical intervention because it affects all age groups and because it can recur in any individual. During the past three decades, efforts to prevent and control influenza have focused primarily on the use of inactivated influenza vaccines in elderly people and in individuals with chronic medical conditions that put them at risk for complications. However, the continuing impact of influenza in these and other population groups has motivated the development of novel approaches for prevention and control of influenza. Several important advances in the field of influenza have occurred in the last few years. An experimental live, attenuated, intranasally administered trivalent influenza vaccine was shown to be highly effective in protecting young children against influenza A H3N2 and influenza B. New antiviral drugs based on the structure of the neuraminidase molecule were assessed in clinical trials and found to be effective against influenza A and B viruses. The expected use of these new antiviral agents has accelerated the development of rapid point-of-care diagnostic tests. The availability of new diagnostic tests, new antiviral drugs, and new vaccines will undoubtedly alter our approaches to influenza control and have an impact on clinical practice.

Influenza is a highly contagious acute respiratory disease of global importance that has caused epidemics and pandemics of human disease for centuries. Most influenza infections are self-limited; however, visits to clinics, physicians' offices, or hospital emergency rooms can increase greatly during epidemics. Lower-respiratory-tract and cardiac complications can lead to substantial increases in hospital admissions and deaths, and health-care resources can be severely strained. Deaths after influenza occur in elderly people and in those with underlying pulmonary and cardiac diseases.[1] Between 1972 and 1992, influenza caused up to 11 800 excess deaths due to pneumonia and influenza, and up to 47 200 excess deaths of all causes during certain influenza seasons in the USA alone.[2] Vaccination with inactivated influenza virus is currently the most effective measure for reducing the impact of influenza; vaccination is among only a small number of cost-effective preventive health interventions for elderly people. In addition, two influenza-A-specific antiviral agents, amantadine and rimantadine, are currently available in several countries. The recommended composition of the influenza virus vaccine is updated annually to provide vaccines antigenically well-matched with new influenza virus strains that are expected to cause epidemics. Only by annual administration of influenza vaccine before the epidemic can health-care providers expect to prevent influenza and its associated complications in high-risk groups. Despite these effective therapies for the prevention and treatment of influenza, annual epidemics continue to cause substantial morbidity and mortality worldwide. Promising new measures for prevention and control of influenza include an intranasally administered live, attenuated influenza vaccine, and a new class of antiviral compounds called neuraminidase inhibitors.

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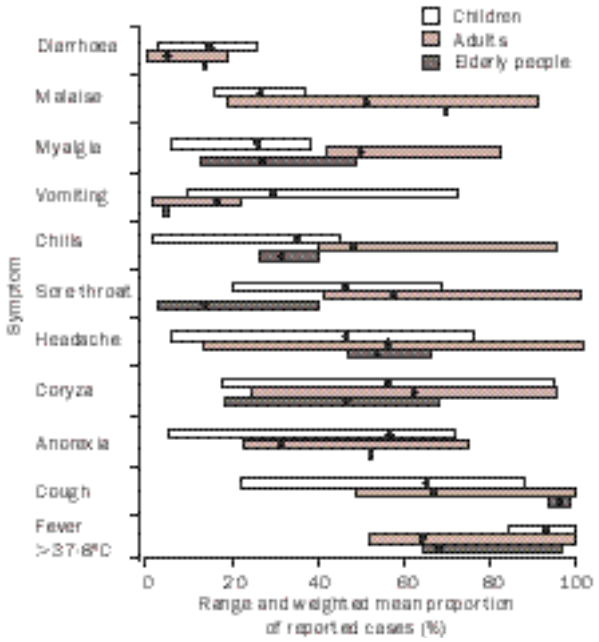
Virology

Influenza viruses are enveloped particles with two surface glycoproteins—haemagglutinin and neuraminidase. Influenza viruses belong to the family Orthomyxoviridae, which includes four genera: influenzavirus A, influenzavirus B, influenzavirus C, and thogotovirus.[3] Influenza A and B viruses contain eight single-stranded, negative-sense RNA segments that encode at least ten polypeptides, of which eight are structural viral proteins, and two are found in infected cells.

Influenza A viruses are further divided into subtypes on the basis of serological and genetic differences in their surface glycoproteins and the genes that encode them. 15 subtypes of haemagglutinin (H1-H15) and nine subtypes of neuraminidase (N1-N9) have been identified. Viruses of all subtypes are present in wild aquatic birds, in which they replicate in the respiratory or intestinal tract, generally without causing disease.[4] Influenza A viruses with haemagglutinin proteins of the H1, H2, and H3 subtypes, and neuraminidase proteins of the N1 and N2 subtypes have caused epidemic and pandemic activity in man since 1900. Various subtypes have also been isolated from pigs, horses, seals, and whales. The substantial reservoir of all known influenza A subtypes in aquatic birds, and the ability of these avian viruses to jump host species barriers, mean that influenza is not considered an eradicable disease.

The epidemiological success of influenza viruses is largely due to two types of antigenic variation that occur primarily in the haemagglutinin and neuraminidase antigens. Such variations render an individual susceptible to new strains, despite previous infection by other influenza viruses. The first type of variation (antigenic drift) occurs in both influenza A and B viruses and is caused by the accumulation of point mutations in the haemagglutinin and neuraminidase genes. Antigenic drift occurs as part of the continuing evolution of influenza viruses. As antibody titres to a newly emerged pandemic or epidemic strain rise within the population, circulating influenza viruses with altered haemagglutinin or neuraminidase antigens become more prevalent by natural selection. Antigenic shift is the second, more



Common symptoms of influenza reported by patients of different ages

profound type of antigenic variation, which occurs only among influenza A viruses. Antigenic shift is defined as the appearance in the human population of a new influenza virus containing a novel haemagglutinin (or novel haemagglutinin and neuraminidase) immunologically distinct from those of the influenza viruses circulating in recent years. Antigenic shift occurs when novel subtypes of influenza that normally infect only birds or pigs are transmitted to man. A pandemic ensues when person-to-person transmission of these novel viruses occurs in a large and immunologically susceptible population. Emergence of novel pandemic strains may occur after genetic reassortment between human and animal influenza viruses, or via direct transmission of an animal strain to people.

Epidemiology

Influenza viruses are unique in their ability to cause both recurrent annual epidemics and more serious pandemics that spread rapidly and may affect all or most age-groups. The size of epidemics and pandemics, and their relative impact, reflects the interplay beween the extent of antigenic variation of the virus, the amount of protective immunity in populations, and the relative virulence of the viruses. Although the epidemiology of influenza has been studied extensively since the virus was first isolated by Wilson Smith and colleagues in 1933,[5] measurements of the extent and impact of influenza are commonly indirect and imprecise. Reporting of individual influenza cases is not generally required because many people who are ill do not seek medical care, and few cases are actually diagnosed. Several rapid tests are now available for laboratory (or clinic) diagnosis of influenza; virus isolation or serological tests can be used to confirm a diagnosis.

Influenza viruses replicate in the columnar epithelial cells of the respiratory tract. From there, they gain access to respiratory secretions and are spread by small-particle aerosols generated during sneezing, coughing, and speaking. Spread of infection by direct contact is also

Age-group and sign / symptom	Mean (range), %
Children	
Otitis media	19 (3–44)
Seizures	16 (6–37)
Croup	16 (4–21)
Conjunctivitis	16 (0–34)
Children and adults	
Pharyngitis	64 (43–83)
Dizziness	24 (3–32)
Hoarseness	21 (3–37)
Abdominal pain	12 (0–35)
Adults	
Arthralgia	42 (19–63)
Chest pain	21 (3–31)
Insomnia	21 (0–38)
Cervical lymphadenopathy	13 (7–16)
Adults and elderly	
Sputum production	39 (32–54)
Elderly	
Dyspnoea	55 (25–60)

Table 1: **Signs and symptoms reported in specific age-groups**[7–20]

possible. It is generally accepted that influenza viruses are maintained in human beings only by direct person-to-person spread. There is no direct evidence for reintroduction of influenza viruses from latently or persistently infected people. The incubation period for influenza (1–4 days) is short, and the explosive nature of influenza epidemics and pandemics, and simultaneous onset in communities, suggests that a single infected person can transmit the virus to a large number of susceptible individuals. This process has been demonstrated in specific circumstances.[6]

Both the H1N1 and H3N2 subtypes of influenza A viruses are circulating currently, along with influenza B viruses. The prevalence of these three groups of viruses may vary temporally and geographically within a country, and between countries and continents during an influenza season. During the past 15 years in the USA, the circulation of influenza A (H3N2) viruses has often been associated with more severe disease and with excess pneumonia and influenza mortality.[2] Antigenic variation and the consequent epidemiological behaviour of influenza follow a fairly uniform pattern, with each successive variant replacing the previous one such that co-circulation of distinct antigenic variants of a given subtype generally occurs for short periods. Intensive surveillance in communities has shown that influenza activity can often be detected during the summer months.

Clinical illness and complications

Influenza in adults and adolescents typically presents with an abrupt onset of fever and chills, accompanied by headache and sore throat, myalgias, malaise, anorexia, and a dry cough. Fever (38–40°C) peaks within 24 h of onset and lasts 1–5 days. Physical signs include the appearance of being unwell, hot and moist skin, flushed face, injected eyes, hyperaemic mucous membranes, and a clear nasal discharge. Although several of the symptoms of influenza are common to all age-groups, a review of published reports of influenza in children, adults, and elderly adults shows that the proportion of patients in whom these complaints are noted varies by age[7–21] (figure). In addition, some clinical signs and symptoms are reported in one age-group but not in another (table 1).

In addition to the illness described above, infants and young children can present with a non-specific febrile

Name	Type of influenza	Principle	Assay method	Time / number of steps	Sensitivity	Specificity
Directigen Flu A	A	Antigen binds to antibody-coated filter	Enzyme immunoassay (immunomembrane filter assay)	15 min / 8 steps	67–96%*	88–97%*
ZstatFlu	A and B	Neuraminidase cleaves chromogenic substrate	Colorimetric neuraminidase enzyme assay	30 min / 4 steps	62%	99%
Biostar	A and B	Antigen binds to antibody-coated wafer, changing optical thickness and altering path of reflected light	Optical EIA	17 min / 7 steps	62–88%*	52–90%*

*Depends on the type of specimen.

Table 2: **Point-of-care rapid diagnostic tests for influenza currently available or in development**

illness, or with a respiratory illness such as croup, bronchiolitis, or bronchitis that is indistinguishable from illnesses caused by other respiratory viral pathogens such as respiratory syncytial virus or parainfluenza viruses. Gastrointestinal complaints are common in children, and include nausea, vomiting, diarrhoea, and abdominal pain. Febrile convulsions are the initial sign in a large number of children. The clinical presentation of influenza in infants can mimic that of bacterial sepsis.[16]

Special situations

Immunocompromised hosts—Anecdotal reports indicate that clinical symptoms of influenza are not unusual in immunocompromised hosts, but that the illness may last longer than normal, and the virus may replicate for weeks to months. Prospective studies are required to define more precisely disease severity and rates of complications in this population.

Pregnancy—Excess influenza-associated mortality in pregnant women was documented during the 1918 and 1957 pandemics,[22] but not for influenza between pandemics. However, Neuzil and colleagues have shown that there is an increased risk of hospital admission for selected cardiorespiratory disorders during the second and third trimesters of pregnancy.[23] As well as the risk to the pregnant woman, there are risks to the fetus; increased rates of miscarriage, stillbirth, and premature birth occurred during the 1918 and 1957 pandemics.[22]

Human H5N1 infections—18 laboratory-confirmed cases of influenza A (H5N1) were identified in Hong Kong in 1997; six patients died. The patients ranged in age from 1 year to 60 years, but the risk of severe illness and death was greater in patients older than 13 years. A report of the clinical features of 12 of the cases shows that all cases presented with a febrile influenza-like illness, eight had upper-respiratory-tract signs, and seven presented with or developed lower-respiratory-tract involvement.[24] In addition to the high case fatality rate, notable features of H5N1 influenza infections were the prominence of gastrointestinal symptoms in adults, and the high rate of complications, including acute respiratory distress syndrome, biochemical evidence of hepatic dysfunction, pancytopenia, reactive histiocytosis with haemaphagocytosis, renal failure, and pulmonary haemorrhage.

Complications

Pulmonary complications—Primary viral pneumonia is associated with a high mortality rate. It begins within 24 h of the onset of febrile illness with a dry cough that later becomes productive of bloody sputum accompanied by tachypnoea, diffuse fine rales, progressive cyanosis, and respiratory failure. Patients deteriorate despite antibiotic therapy. Bilateral interstitial infiltrates can be seen on chest radiography, but influenza virus infection can cause radiological changes similar to those of other causes of pneumonia. Secondary bacterial pneumonia is characterised by the appearance of a new fever and productive cough during early convalescence. Clinical signs of lobar consolidation can be confirmed radiologically. *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Haemophilus influenzae*, and group A β-haemolytic streptococci are the bacterial pathogens most commonly identified. Patients with secondary bacterial pneumonia respond to antibiotic therapy. A combined viral and bacterial pneumonia can present with any combination of signs and symptoms, and shows a variable response to antibiotic treatment. Influenza can lead to an acute exacerbation of chronic bronchitis in people with chronic obstructive pulmonary disease or cystic fibrosis, and to wheezing in patients with asthma.[25–27]

Neurological complications—Reye's syndrome, an acute encephalopathy with cerebral oedema (with raised cerebrospinal fluid [CSF] pressure but normal CSF cell counts and chemistry) and fatty degeneration of the liver, has been reported in patients using aspirin after influenza infections. Other complications involving the central nervous system include impaired reaction times, postinfluenza encephalitis, encephalopathy, transverse myelitis, and Guillain-Barré syndrome.[7] Two studies have reported the detection of influenza virus RNA in the CSF of patients with encephalopathy associated with an influenza-like illness.[28,29]

Other complications—In addition to its association with pneumonia, *Staphylococcus aureus* is seen in cases of toxic shock syndrome after influenza.[30] Myositis, which is more common in children than adults, and is more commonly associated with influenza B than with influenza A virus infections, presents in early convalescence with an acute onset of pain and tenderness in the gastrocnemius and soleus muscles that can be severe enough to limit walking. Serum creatine phosphokinase concentrations are transiently raised. Complete recovery generally occurs in 3–4 days. Myoglobinuria and renal failure can occur rarely. Cardiac muscle damage with associated electrocardiographic changes, disturbances of rhythm, and high concentrations of cardiac enzymes have been reported after influenza virus infection.

Laboratory diagnosis

A definitive diagnosis of influenza requires laboratory confirmation. There are several new diagnostic tests for influenza available or soon to be available. Diagnostic tests for influenza fall into four broad categories: virus isolation, detection of viral proteins, detection of viral nucleic acid, and serological diagnosis. The best clinical sample to use with the first three diagnostic methods is a combination of nasopharyngeal and throat swabs. The importance of appropriate collection and handling of clinical samples cannot be overemphasised for all types of diagnostic test.

Virus isolation is the gold standard for laboratory diagnosis of influenza; the virus is available for genetic and antigenic analysis, but results are not usually available rapidly enough to be the basis for initation of antiviral therapy or infection-control measures. Several clinical laboratories use a rapid culture method, whereby the clinical sample is centrifuged onto a monolayer of cells, which are fixed at 24–48 h, and stained for viral antigens by immunofluorescence. Although the time to obtain a result is much shorter, the usefulness of rapid cultures may still be limited for a clinician considering antiviral therapy.

Detection of viral proteins is an area of exciting developments (summarised in table 2). These diagnostic techniques are rapid and easy to perform; some are designed for use at the point-of-care. These tests are just appearing on the market and have not yet been compared with one another. The tests are less sensitive than culture or PCR, but results are available in less than 1 h, and can be used to guide use of antiviral agents.

Detection of viral nucleic acid (RNA) in clinical material is possible by reverse-transcription followed by PCR with gene-specific oligonucleotide primers. Such primers can be selected to type and subtype influenza viruses, and can also be combined with specific primers for other viral agents, such as respiratory syncytial virus, in a multiplex PCR format.[31] This method is very sensitive; laboratories may choose among several methods to confirm PCR results. PCR can detect non-viable virus, and great care must be taken in the laboratory to avoid contamination of specimens.

Serological diagnosis of influenza is based on the detection of a four-fold or greater rise in specific antibody titre in paired serum samples, measured by haemagglutination inhibition, EIA, complement fixation, or neutralisation tests. The need for paired serum samples, the first collected as soon as possible after onset of illness and the second collected 10–14 days later, limits the usefulness of serology in diagnosis and treatment of acute illnesses.

For the clinician, each approach has benefits and limitations. The choice of a test is likely to be affected by factors such as cost, sensitivity, and specificity. Limited data are available about the use of newer diagnostic tests on bronchoalveolar lavage fluids and tracheal aspirates. Virus isolation must not be abandoned because antigenic and genetic characterisation of virus isolates forms the basis for the selection of virus strains to be included in vaccine formulations.

Control and prevention

The two approaches available for the control and prevention of influenza are the use of vaccines and the use of antiviral agents.

Vaccines

The haemagglutinin and neuraminidase proteins are the primary targets of the protective antibody response; antibodies against haemagglutinin neutralise virus infectivity, and antibodies against neuraminidase can modify the severity of disease. Influenza vaccines elicit a strain-specific antihaemagglutinin immune response. Serum haemagglutination inhibition antibody titres are the most commonly measured correlate of protection; susceptibility to infection is inversely related to these titres, and postvaccination titres of about 1 to 40 in serum represent the level of antibody at which about 50% of the population will be protected.[32]

Specific recommendations for vaccine use are made by national authorities, and the vaccine is generally recommended for use in people with factors that predispose them to severe morbidity and mortality. Currently licensed influenza vaccines are trivalent inactivated formulations that contain 15 μg each of the haemagglutinin of influenza A (H1N1), influenza A (H3N2), and influenza B strains. The inactivated vaccine is safe and immunogenic, inducing immunity in 60–90% of children and adults; however, immunogenicity is generally lower in elderly people. The efficacy of the vaccine ranges from 70% to 90% in healthy young adults but may be lower in other populations.

In a study of children, influenza vaccination decreased the incidence of acute otitis media during influenza season.[33] In another, of elderly people, immunisation was cost effective and associated with reductions in rates of hospital admission and deaths from influenza-related complications.[34] In an assessment of influenza vaccination in healthy, working adults, vaccination was associated with fewer episodes of upper respiratory illness, fewer days of sick leave, and fewer visits to physician's offices for upper respiratory illnesses.[35]

The inactivated vaccine is generally well tolerated; however, because the vaccine is grown in eggs, it is contraindicated in people with serious egg allergies.[36] James and colleagues[37] reported that influenza vaccines containing less than 1·2 mg/mL egg protein could be used safely in a two-dose protocol in individuals with a history of egg allergy. Although HIV-infected individuals show rises in antibody titres to influenza after immunisation with inactivated vaccine, reports about the effect of influenza immunisation on HIV-1 viral load are contradictory.[36] The 1976 influenza vaccine produced to combat the (H1N1) swine influenza virus was associated with increased incidence of Guillain-Barré syndrome, but any association between this syndrome and subsequent vaccine strains has been less clear.[36] In a study of the 1992–93 and 1993–94 influenza seasons combined, Lasky and colleagues[38] reported an overall relative risk for Guillain-Barré syndrome of 1·7 (95% CI 1·0–2·8) during the 6 weeks after vaccination; this risk represents an excess of slightly more than one additional case of Guillain-Barré syndrome per million people vaccinated, and is substantially lower than the risk of severe influenza. Influenza can be prevented by vaccination in all age-groups, especially people aged 65 years or older, and those who have medical indications for influenza vaccination.[36,38]

Some of the disadvantages of the inactivated vaccine are poor induction of mucosal IgA antibody and cell-mediated immune responses, and lower immunogenicity and efficacy in the elderly. Mucosal delivery and adjuvants are being investigated to improve these properties.

Intranasally administered live, attenuated, cold-adapted influenza virus vaccines replicate in the upper respiratory tract, and elicit a specific protective immune response; such vaccines are in use in Russia and are under development in the USA. The vaccines (derived by genetic reassortment between appropriate wild-type influenza viruses and the cold-adapted master donor strains) bear the haemagglutinin and neuraminidase genes of the wild-type viruses, and attenuating internal

gene segments of the master donor strains. The two master donor strains that have been assessed in the USA are A/Ann Arbor/6/60 (H2N2) and B/Ann Arbor/1/66.

Clinical trials have established the safety, immunogenicity, and efficacy of the cold-adapted live attenuated vaccines.[39] Vaccine efficacy, measured as protection against culture-positive influenza, in children who received two doses of vaccine was 96% (95% CI 90–99) against influenza A (H3N2) and 91% (78–96) against influenza B.[40] In healthy working adults, live, attenuated influenza vaccine decreased febrile upper respiratory tract illness, days of work lost due to febrile respiratory illness, health-care-provider visits, and use of antibiotics and over-the-counter medications.[41] Limited clinical studies in elderly people suggest that these patients may benefit from a combination of inactivated and live attenuated vaccines.[39]

The advantages of the intranasally administered live vaccines are the ability to elicit a mucosal immune response (in addition to a humoral and cellular systemic immune response), ease of administration, and acceptability. A live virus vaccine may also elicit a longer-lasting or broader immune response than inactivated vaccines; clinical evidence must be gathered to support this hypothesis.

Antiviral drugs

Two anti-influenza A drugs are currently licenced in some countries. They are the chemically related adamantane compounds, amantadine and rimantadine—both of which are 70–90% effective in preventing illness caused by naturally occurring influenza A viruses when administered prophylactically to healthy adults or to children during the period of exposure in a normal epidemic or outbreak situation. When used therapeutically within 48 h of the onset of symptoms, these two compounds can also reduce the severity and duration of signs and symptoms of illness caused by influenza A viruses. Although the effectiveness of the two compounds is similar, rimantadine has a better safety profile. Amantadine is excreted renally and can cause substantial neurological side-effects, particularly in individuals whose kidney function is impaired, including elderly people.[36] In the USA, these antiviral compounds are recommended for prophylactic use in individuals at high risk who have not been vaccinated; in individuals with immunodeficiency; in people who have severe anaphylactic hypersensitivity to egg protein or other vaccine components; in residents of institutions such as nursing homes for outbreak control; and in hospital personnel and others providing care to those at high risk.[36] Both drugs interfere with the replication of influenza A (but not influenza B) viruses through blocking the function of the M2 protein—a membrane-spanning protein essential for uncoating of the virus after entry into the host cell. Amantadine-resistant viruses with mutations in the M2 protein are cross-resistant to rimantadine, and vice versa. Drug-resistant viruses have been isolated from patients when either amantadine or rimantadine is used for therapy; however, the frequency with which resistant viruses are transmitted, and their impact on efforts to control influenza are unknown.

The sialic acid analogues specifically inhibit both influenza type A and B neuraminidase—the viral enzyme that cleaves terminal sialic acid residues from glycoconjugates to allow the release of virus from infected cells, to prevent the aggregation of virus, and possibly to reduce viral inactivation by respiratory mucus. Two neuraminidase inhibitors, Relenza (zanamavir or GG 167; administered by inhalation) and Tamiflu (oseltamivir or GS4104; administered orally) have been tested in phase III clinical trials, with promising results.[42–46] When administered within 30–36 h of onset of illness, zanamavir shortened the time to alleviation of major influenza symptoms by 1–2 days, but the drug provided no benefit to people without laboratory-confirmed influenza.[42–44] Zanamivir has also been shown to be safe and effective in preventing influenza in healthy adults.[45] Both compounds are active against influenza A and B viruses, and seem less likely to induce the development of resistant viruses than do adamantanes. The only documented case in which zanamavir resistance was noted thus far developed after long-term treament of an influenza B virus infection in an immunocomprised child.[47] Further studies of these drugs in high-risk individuals, and comparisons with adamantanes are in progress. Neuraminidase inhibitors have now been approved in some countries, and their approval is expected soon in others.

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