

Viral–bacterial interactions–therapeutic implications

Jane C. Deng

Division of Pulmonary and Critical Care Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA.

Correspondence: Jane C. Deng, Division of Pulmonary and Critical Care, UCLA, 10833 Le Conte Avenue, 37-131 CHS, Los Angeles, CA 90095, USA

E-mail: jdeng@mednet.ucla.edu

ABSTRACT

Viral and bacterial respiratory tract infections are a leading cause of morbidity and mortality worldwide, despite the development of vaccines and potent antibiotics. Frequently, viruses and bacteria can co-infect the same host, resulting in heightened pathology and severity of illness compared to single infections. Bacterial superinfections have been a significant cause of death during every influenza pandemic, including the 2009 H1N1 pandemic. This review will analyze the epidemiology and global impact of viral and bacterial co-infections of the respiratory tract, with an emphasis on

bacterial infections following influenza. We will next examine the mechanisms by which viral infections enhance the acquisition and severity of bacterial infections. Finally, we will discuss current management strategies for diagnosing and treating patients with suspected or confirmed viral-bacterial infections of the respiratory tract. Further investigation into the interactions between viral and bacterial infections is necessary for developing new therapeutic approaches aimed at mitigating the severity of co-infections.

Keywords bacterial pneumonia, influenza, polymicrobial infections, respiratory viral infection.

Please cite this paper as: Deng (2013) Viral–bacterial interactions–therapeutic implications. *Influenza and Other Respiratory Viruses* 7(Suppl. 3), 24–35.

Introduction

Influenza and pneumonia are the leading cause of death from an infectious disease in the United States and worldwide.¹ They consistently rank among the top 10 causes of death in the United States, resulting in approximately 50 000 deaths each year.² Worldwide, an estimated 3.5 million people succumb to these infections annually, more than HIV/AIDS and tuberculosis combined.¹ Because pinpointing the precise microbiologic cause of a respiratory infection is impractical or infeasible in many cases, it is often presumed that pneumonias are caused by either bacteria or respiratory viruses like influenza, and when in actuality, many lower respiratory tract infections are caused by multiple pathogens acting in synergy. Notably, bacterial pneumonias have been long appreciated to be a major complication of influenza infections, even after the advent of antibiotics. During the 2009 pandemic, bacteria pneumonias were present in 25–30% of severe cases requiring hospitalization, and up to 50% in small autopsy series, thus demonstrating the continued public health significance of influenza–bacterial co-infections despite the availability of vaccines and potent antibiotics.^{3–7}

In this review, I will discuss the types of co-infections that are often encountered in clinical practice. The focus will be primarily on co-infections of the respiratory tract involving acute respiratory viruses and community-acquired bacterial pathogens, particularly secondary bacterial pneumonia

following influenza. First, I will discuss the epidemiology of co-infections. This will be followed by a brief overview of findings from research into the basic mechanisms underlying the pathogenesis of viral–bacterial co-infections. Finally, I will present recommendations on how these findings can be utilized clinically, as co-infections clearly pose a therapeutic challenge for clinicians and public health officials alike.

What types of co-infections are encountered in practice?

Viral–bacterial co-infections are regarded to be a common and clinically significant problem, although the precise incidence is difficult to determine for a variety of reasons. First, many cases of viral–bacterial co-infections go undetected, particularly in the outpatient setting. A common scenario is a patient who presents to a clinic with a history of a (presumably viral) upper respiratory infection (e.g., nasal congestion, rhinorrhea, sore throat) which had improved initially, but then worsened a few days later and now has complaints that are suspicious for bacterial infection (e.g., productive cough, sinus pain, fevers). In these cases, an earnest search for the microbiologic etiologies is often not warranted. Second, even when further testing is performed, the diagnostic yield is limited by the sensitivity of currently available microbiologic tests and the ability to obtain certain types of clinical specimens (e.g., sputum, bronchoalveolar lavage). Furthermore, the early administration of antibiotics,

while appropriate for clinical management of acutely ill individuals, can considerably diminish the yield of bacterial cultures. For these reasons, interest has turned to adjunctive molecular testing methods, such as nucleic acid amplification tests (NAATs) [e.g., viral respiratory polymerase chain reaction (PCR) panels] or direct antigen detection methods (e.g., for *Streptococcus pneumoniae*, *Legionella pneumophila*, influenza, etc.), which can improve the overall diagnostic yield but lack specificity. Thus, unless a study was performed as part of a large clinical trial with comprehensive protocols for diagnostic testing or the medical facility has strict diagnostic algorithms in place for workup of pneumonia, the true incidence of viral–bacterial co-infections is difficult to determine and likely underestimated.

Despite these issues, both upper and lower respiratory tract illnesses are frequent complications of viral infections. Acute otitis media in pediatric populations are often observed during viral outbreaks, most commonly respiratory syncytial virus (RSV), adenovirus, human rhinovirus (HRV), and coronavirus.^{8–11} A recently identified virus isolated from the human respiratory tract, human bocavirus,¹² has emerged as a potentially important copathogen in acute otitis.¹³ It is believed that changes in the upper respiratory tract induced by antecedent viral infections are what facilitate bacterial invasion into the middle ear. The bacteria, which most frequently cause otitis, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, and non-typeable *Hemophilus influenzae*, are normal members of the nasopharyngeal flora; viral infections appear to enable these bacteria to enter the middle ear—and more importantly, flourish—resulting in disease.¹⁴ Substantial variation exists in the patterns of viral–bacterial co-infections reported, depending on the patient population (e.g., location), prevalence of vaccination against pneumococcus, and method of microbiologic detection (e.g., middle ear aspirate, nasopharyngeal swabs) The viral–bacterial combinations most strongly associated with acute otitis media include rhinovirus or RSV with *M. catarrhalis*, *S. pneumoniae*, or *H influenzae*;^{10,13,15,16} however, this may also be attributable to the fact the RSV and rhinoviral infections are very common in the pediatric population.

Pneumonia and other lower respiratory tract infections follow a pattern similar to otitis, in that increased rates of pneumonia appear to coincide with the time periods when respiratory viruses are more prevalent, particularly influenza and RSV.^{17,18} The association between influenza and bacterial pneumonia has long been recognized.¹⁹ During the late 1800s and early 1900s, the bacterium now known as *H. influenzae* was so commonly isolated from patients with influenza infection that it was believed to be the etiologic agent causing the 1918–1919 pandemic, since at that time, viruses had barely been discovered. Retrospective analysis of specimens from the 1918 influenza pandemic has revealed that almost all fatal cases of pneumonia showed evidence of

bacterial infection.¹⁹ Since then, epidemiologic studies during influenza pandemics and epidemics have demonstrated incidence of pneumonias peaking concurrently with influenza activity.^{20–22} Although evidence of bacterial infection was not present in all cases, when bacterial cultures were positive, it was almost always *S. pneumoniae*, *S. aureus*, *S. pyogenes*, *H. influenzae*, or a combination of these bacteria.^{21–24} Generally, secondary bacterial pneumonia complicating influenza infection has been noted to be more severe and prolonged, with higher mortality rates.^{22,23} However, some influenza seasons are characterized by lower rates of mortality demonstrating that viral factors (e.g., the influenza neuraminidase) other currently unknown factors are responsible for determining the incidence and severity of influenza–bacterial co-infections.^{24–28} Influenza seasons where H3N2 influenza A dominate appear to be weakly associated with higher rates of invasive pneumococcal infections, which may be attributable to the higher neuraminidase activity of H3N2 strains compared with H1N1.^{27,29} Severe tracheobronchitis caused by less virulent bacteria is another frequently noted complication of influenza.²²

Although the mortality rates from bacterial pneumonia following influenza have significantly declined over the 20th century for a variety of reasons, including changes in the epidemiology of influenza viruses, development of antimicrobial therapies and vaccines, and improvements in supportive care, bacterial pneumonias remain an important contributor to the severity and lethality of influenza infections.^{28,30} During the recent 2009 H1N1 influenza pandemic, bacterial pneumonia was present in 4–33% of hospitalized or critically ill patients.^{3,7,31–34} Pathologic and microbiologic analyses of fatal cases showed evidence of bacterial co-infections ranging from 25% to 55%.^{4–6,35–37} Microbiology data of secondary pneumonias from some of these studies are presented in Table 1. Adding to the body of evidence for the continued importance of influenza and bacterial co-infections are epidemiologic studies reporting excess hospitalization rates for pneumococcal pneumonia during the 2009 H1N1 pandemic.^{38,39} A recent analysis of population-level data in the United States during the 2009 pandemic and non-pandemic years revealed a spike in invasive pneumococcal pneumonia rates that coincided with influenza activity.⁴⁰ Furthermore, a recent study conducted in the United States of critically ill adults with 2009 influenza A infection reported that patients with bacterial co-infections had 50% higher mortality compared to patients without.⁷

The continued intense focus on influenza pandemics, however, has somewhat obscured the fact that bacterial pneumonias frequently complicate other respiratory viruses. Invasive pneumococcal disease has been shown to be as strongly correlated with RSV as influenza, particularly among children.^{41–44} This may, in part, reflect the higher prevalence of RSV infections among children with symptomatic lower

Table 1. Epidemiology of bacterial co-infections during 2009 H1N1 influenza pandemic

First Author	Study type	Location	Total # cases	# bacterial co-infection (%)	Number of secondary bacterial infections				Ref #
					Sp	Sa	Spy	Other	
Mauad Shieh	Autopsy	Brazil	21	8 (38%)	6			2 N.D.	6
	Autopsy	U.S.	100	33 (33%)	9	8 (4 MSSA, 4 MRSA)	3	4 mixed 1 <i>S. mitis</i> 1 <i>S. agalactiae</i> 7 N.D.	35
Lee	Fatalities	New York City	47	13 (28%)	8		3	1 mixed 1 N.D.	5
Gill	Autopsy	New York City	33	18 (55%)				16 Streptococcal, 2 Staphylococcal	4
	Gram Stain							2 Staphylococcal	
Cox	Postmortem culture/IHC		30	10 (33%)	6/30	1 MRSA	2	1 mixed (Sp+Spy)	37
	Pediatric deaths	U.S.	317	46 (28%)	12	18 (5 MSSA, 11 MRSA, 2 unknown)	4	5 other <i>Streptococcus</i> sp. 6 gram-negative bacteria (some patients polymicrobial)	
Viasus	Hospitalized adults	Spain	585	36 (6%)	26	4 MSSA	1	2 <i>H. influenzae</i>	140
Martin-Loeches	ICU patients >age 15	Spain	645	113 (17.5%)	62/113	9 MSSA	6/113	1 <i>H. parainfluenzae</i> 2 <i>Acintebacter baumannii</i> 1 <i>Pseudomonas aeruginosa</i> 1 <i>Moraxella catarrhalis</i> 2 <i>Streptococcal</i> sp. (4 polymicrobial)	33
								10 <i>Aspergillus</i> 9 <i>Pseudomonas aeruginosa</i> 4 <i>Acintebacter baumannii</i> 4 <i>Klebsiella pneumoniae</i> 4 atypical 3 <i>H. influenzae/Moraxella</i> 2 other	
Rice	Adult ICU patients	U.S.	683	207 (30%) based on clinical suspicion	17*	47* (18 MSSA, 29 MRSA)		4 Group A* <i>Streptococcus</i> 53 N.D. (respiratory and blood)	7
Randolph	Pediatric ICU patients	U.S.	838	274 (33%) based on clinical suspicion	15	71 (37 MSSA, 34 MRSA)	7	30 <i>Pseudomonas</i> 14 <i>Moraxella catarrhalis</i> 13 <i>H. influenzae</i> 95 Other 91 N.D. (some patients polymicrobial)	34

N.D., not detected/not done.
IHC, immunohistochemistry.

Sp, *S. pneumoniae*; Sa, *S. aureus*; Spy, *S. pyogenes*.

*Results of respiratory culture within 72 hours of admission only.

respiratory infections.^{17,18} A number of studies have described the patterns of viral–bacterial co-infections in children hospitalized with community-acquired pneumonia (CAP).^{45–48} In this population, rates of viral–bacterial co-infections usually range from 15% to 30%, with higher rates in the younger age groups (under 5 years of age). The most common causes of CAP and acute lower respiratory tract infections (LRTI) in children are *S. pneumoniae*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and respiratory viruses, although in a large part of the world, *H. influenzae* and *S. aureus*, remain important causes. Thus, the types of viral–bacteria co-infections tend to vary by age group and geography, but generally represent the pathogens most commonly isolated in the study population (e.g., RSV–*S. pneumoniae*, rhinovirus–*Mycoplasma*) although almost any combination of respiratory virus–bacterial pathogen has been observed. Although the widespread use of vaccination against *H. influenzae* type b has largely eliminated this as a cause of CAP in the United States and other high-income countries, this pathogen causes an estimated 4–1% of severe and 15–7% of fatal cases of childhood pneumonias worldwide.⁴⁹ In adults, viral–bacterial co-infection rates among patients with CAP are around 4–16% and are associated with more severe disease.^{50,51} Influenza, rhinovirus, RSV, and adenovirus are the most common viruses isolated from patients with bacterial CAP.⁵⁰ These epidemiologic studies are a reminder that viruses are an important etiologic agent of LRTIs, as well as a major cofactor in the development of severe bacterial pneumonia. Hence, clinicians should counsel patients who present with an initial respiratory viral infection of the potential risk of developing secondary bacterial infections, and to return if symptoms worsen. Conversely, clinicians caring for patients who present with evidence of severe lower respiratory tract infections when influenza is known to be circulating should consider the epidemiology of bacterial pathogens in this setting and consider empiric treatment of both influenza and *S. aureus* in addition to the usual regimen for community-acquired pneumonia.

How do viruses predispose individuals to bacterial infections?

Evidence from our laboratory and many other investigators have shown that enhanced susceptibility to bacterial pathogens (e.g., *S. pneumoniae*, *S. aureus*) peaks anywhere from 4 to 14 days after the primary influenza infection, but can persist to 30 days and beyond.^{52–54} In a murine model of sequential influenza and *S. pneumoniae* infection, we have found that at 48 hours after bacterial challenge, influenza-infected mice infected with only 200 colony-forming units (CFU) of *S. pneumoniae* have comparable lung bacterial burdens as non-influenza-infected animals challenged with 10⁶ or 10⁷ CFU, underscoring the profound immune defects

Table 2. Pathogenetic mechanisms of viral–bacterial co-infections.

Virus induced alteration in epithelial cells
Reduced ciliary function
Cell death/decreased epithelial barrier function
Upregulation of surface receptors for bacterial adhesion
Enhancement of bacterial colonization and transmission <i>in vivo</i>
Virus-mediated inhibition of innate immune cells (e.g., macrophages, neutrophils)
Suppressed phagocytosis
Impaired microbial killing
Depressed leukocyte migration
Antiviral immune molecules – Type I and II interferons
Suppressed innate immunity
Inhibition of IL-17 responses
Dysregulated inflammation
Enhanced lung injury from increased inflammation (e.g., chemokines)
Increased susceptibility from induction of anti-inflammatory cytokines (e.g., IL-10)

induced by influenza. (Jane Deng, unpublished observations) A number of mechanisms likely contribute to the impairments in host defense of the respiratory tract against bacteria following viral infection. Much of our understanding has arisen from studies conducted in animal models of sequential infections by influenza and various bacterial pathogens and has been comprehensively reviewed elsewhere;^{28,55} hence, only the fundamental themes will be highlighted here. (Table 2)

First, influenza and other respiratory viruses, due to their tropism for epithelial cells, induce structural alterations in the respiratory epithelium resulting in improved access to the lower respiratory tract and persistence of upper airway bacteria. Influenza viruses reduce human and animal nasal and tracheal epithelial ciliary function.^{56,57} Influenza and other respiratory viruses can induce death of epithelial cells, leading to compromised barrier function of the airway, and promote adhesion of bacteria through various mechanisms including upregulation of surface receptors such as platelet-activating factor receptor, which is involved in pneumococcal invasion.^{52,58–61} However, the relative importance of this mechanism is debated, given that most respiratory viruses do not induce histologic evidence of significant epithelial damage, and *in vivo* evidence has been inconclusive.^{52,61}

Influenza viruses also enhance colonization and transmission of bacteria. Studies in mice demonstrate that the rate and duration of pneumococcal colonization are enhanced by influenza infection, which may be mediated by a type I interferon-dependent mechanism.^{62–64} In addition, transmission of bacteria to uninfected contacts was markedly enhanced by influenza viral infection of donor and recipient animals. Similar findings have been shown in ferrets.⁶⁵

Viruses also have multiple effects on immune cells, including innate leukocytes critical to antibacterial host

defense such as macrophages and neutrophils. Macrophages are the main resident innate immune cell in the airspaces of the lung and act as immune sentinels for bacterial infections. Influenza, RSV, and other viruses have been shown to suppress macrophage and monocyte chemotaxis and function, including phagocytosis and microbial killing.^{66–73} Similarly, RSV and influenza have been shown to depress neutrophil migration, phagocytosis, and bactericidal activity.^{74–81} On a molecular level, one potential explanation for some of these effects is sustained macrophage desensitization to bacterial Toll-like receptor ligands following influenza and RSV exposure, which results in decreased neutrophil recruitment.⁸² Given the ample evidence that both cell types are essential to the clearance of bacterial pathogens from the lung, it is likely that influenza-induced suppression of phagocytic cell populations plays a major role in governing susceptibility to secondary bacterial infections.

Another emerging theme is that molecules important to antiviral immunity may be detrimental when the influenza-infected host encounters a secondary bacterial pathogen. Activation of type I interferons (e.g., IFN α and β), type II interferon (IFN- γ), and innate immune receptors responsible for recognizing influenza and other respiratory viruses [e.g., Toll-like receptor (TLR)-3, Retinoic acid inducible gene (RIG)-I] have all been shown to mediate susceptibility to secondary bacterial infections.^{53,54,83–85} Among the effects of these immune pathways are impairment in phagocytic cell function and recruitment, and inhibition of the IL-17 pathway possibly through IFN-induced suppression of gamma-delta T cells. Although these mechanisms have mainly been studied in the context of primary influenza infection, we have found that simply inducing type I IFNs in the lung by intranasal administration of poly I:C (ligand for TLR3 and RIG-I) is sufficient to impair bacterial clearance of *S. pneumoniae* and MRSA, suggesting that these mechanisms may be operational in mediating susceptibility to bacteria following infection by other respiratory viruses.⁸⁵ In addition, this also raises the possibility that a host may experience a mild or subclinical infection sufficient to induce an antiviral immune response, thereby decreasing the threshold of susceptibility to bacterial infection. These findings will need to be confirmed in human studies, but should raise some concern that efforts focused on augmenting antiviral immune responses as a strategy for treating pandemic influenza may paradoxically promote the development of secondary bacterial infections.

Finally, dysregulated inflammation is an integral contributor to the pathogenesis of co-infections. On the one hand, an overly robust inflammatory response is believed to underlie the lung injury of highly pathogenic influenza viruses as well as influenza–bacterial co-infections.^{86–91} On the other hand, an imbalance of anti-inflammatory mediators may increase host susceptibility to secondary bacterial

pneumonia. Interleukin-10 is an anti-inflammatory immune molecule that is critical for regulating excess pulmonary inflammation during influenza;⁹² however, elevated IL-10 levels are detrimental to bacterial clearance during secondary bacterial pneumonias.^{93,94}

Collectively, these studies illustrate the considerable complexity in understanding the pathogenesis of polymicrobial infections, which has hampered the development of therapies aimed at restoring antibacterial defense following influenza infections. At present, immunomodulatory therapies aimed at reducing the risk of secondary bacterial infections are largely at the pre-clinical stage of development. However, based upon our current knowledge, a balance must be struck between maintaining adequate antiviral immunity without compromising antibacterial responses, while ensuring that excess inflammation does not lead to lung injury.

Management of patients with viral–bacterial co-infections

Vaccination Reducing the burden of disease from viral–bacterial co-infections starts with an effective vaccination program. Currently, effective vaccines exist for *H. influenzae* type b, *S. pneumoniae*, and influenza, but efforts to develop a vaccine against *S. aureus* have been unsuccessful so far. In addition, there are presently no vaccines for group A streptococcal species, RSV, or many other acute respiratory viruses. Furthermore, vaccination rates remain suboptimal in large parts of the world. By 2011, the *H. influenzae* type b (Hib3) vaccine had been introduced in 177 countries (91%); however, vaccine coverage varied widely by region, ranging from 11% in South-East Asia to 90% in the Americas.⁹⁵ The pneumococcal vaccine had been introduced in only 72 countries (37%) as of 2011, with ongoing efforts by the World Health Organization (WHO) and the Global Alliance for Vaccines and Immunization to expand vaccine availability globally. Presently, the countries that carry the largest burden of bacterial pneumonia have the lowest vaccination rates and therefore are at greatest risk of secondary pneumonias during influenza pandemics.

There are presently two types of vaccines for *S. pneumoniae*—the polyvalent conjugate vaccine (PCV13), which is directed against 13 serotypes and recommended in children (ages 2 years to 59 months), and the polysaccharide vaccine (PPSV23) based on the 23 most common capsular serotypes and recommended in adults 65 years and older, as well as individuals between 2 and 64 years old with risk factors for pneumococcal disease. Both have been shown to be effective at reducing invasive pneumococcal disease caused by the vaccine strains (e.g., bacteremia), although disease caused by non-vaccine strains is becoming more common. Interestingly, cohort and case–control studies in the United States and elsewhere have reported that pneumococcal vaccination

reduces hospitalization with confirmed respiratory viral infections, including influenza and human metapneumovirus, suggesting that pneumococcal infections are a significant cofactor in acute respiratory viral infections.^{96–99}

Influenza vaccination is recommended annually for everyone over 6 months of age. Observational studies suggest that influenza vaccines reduce hospitalization rates for pneumonia and influenza in the elderly and otitis media in children.^{100–105} However, more definitive evidence that influenza vaccines reduce complications such as bacterial co-infections will be difficult to come by, as randomized trials comparing vaccination to placebo group might be viewed as unethical.

Diagnostic testing

Distinguishing viral from bacterial pneumonia can be difficult, even with ample resources. Influenza and other respiratory viruses can cause lower respiratory tract disease. For example, a retrospective analysis of CT scan findings in patients with LRTIs demonstrated considerable overlap in findings between those of viral and bacterial origins.¹⁰⁶ However, diffuse airspace disease was commonly associated with bacterial infection, whereas viral infections tended to have a more airway-centric pattern of involvement, such as “tree-in-bud” findings. Although productive cough, fever, and chills are classical findings in bacterial pneumonia, they are often present in patients with influenza and other respiratory viral infections, and certainly would not enable the clinician to determine whether someone had viral–bacterial co-infections. Rapid microbe-based point-of-care tests such as the rapid influenza antigen tests are limited due to their relatively low sensitivity. Hence, much attention has been turned toward finding biomarkers specific for bacterial infections. Not only are such tests important at the individual patient level, but also from a global health perspective, as the information can aid clinicians in decreasing unnecessary antibiotic use, thereby reducing the risk of antibiotic resistance.

Two biomarkers that have received much attention are C-reactive protein (CRP) and procalcitonin (PCT).^{107,108} Both can be detected in the blood, making them more attractive than other biomarkers that are mainly elevated in bronchoalveolar lavage fluid, such as soluble triggering receptor on myeloid cells (sTREM).^{109,110} In general, levels of CRP and PCT tend to be higher in patients with bacterial infections, compared with viral infections, which may aid clinicians in determining whether patients have a bacterial superinfection during influenza season.¹¹¹ Randomized trials suggest that CRP or PCT-guided management can result in reduced antibiotic usage,^{112–118} which may aid in prioritizing patients for treatment when antibiotics are in short supply. However, no single test can completely distinguish bacterial infections from other infectious causes of pneumonia, as

there is considerable overlap in levels between of patients with and without bacterial disease.¹¹⁹ Furthermore, the overlap in PCT or CRP levels between viral and bacterial infections is more pronounced in malaria-endemic areas, further limiting the ability of these biomarkers to distinguish one type of infection from another.¹²⁰ The clinical utility of biomarkers continues to be investigated.

Treatment

Various respiratory and infectious disease societies from around the world have issued guidelines for treatment of LRTIs, including community-acquired pneumonia (CAP).^{121–130} Treatment of LRTIs caused by viral–bacterial co-infections is governed by regional epidemiologic patterns of respiratory viruses and bacteria. *S. pneumoniae* and *H. influenzae* are the most prevalent causes of community-acquired pneumonia worldwide, the latter occurring primarily in developing countries where the Hib vaccine is not widely available. Atypical pathogens, such as *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, and *Legionella pneumophila*, are collectively considered to be common causes of LRTIs from epidemiologic studies carried out in Europe and other developed countries, but the exact incidence is difficult to determine in areas of limited healthcare resources due to the need for special laboratory testing.^{131–133} Specific treatment regimens recommended by the guidelines often reflect local antibiotic resistance patterns, so guidelines that are suitable for one region—or even a country within that region—may be inappropriate for another. For example, the United States and parts of Europe have rates of penicillin-non-susceptible (i.e., resistant or intermediate susceptibility) *S. pneumoniae* that exceed 20%, whereas Sweden and other northern European countries have non-susceptibility rates of <5%.^{134,135} Hence, penicillin can be used as first-line monotherapy in the treatment of CAP in Sweden,¹³⁰ while a fluoroquinolone or beta-lactam in combination with a macrolide is recommended in the U.S. pneumonia guidelines for hospitalized adults.¹²⁴

In addition to microbiologic considerations, antibiotic treatment decisions are based upon severity of presentation (i.e., risk of death). Commonly used scoring systems for assessing risk level of CAP are the CURB-65 (Confusion, Urea, Respiratory Rate, Blood Pressure, and Age>65) and the more complicated pneumonia severity index (PSI), which include patient characteristics (e.g., age, comorbidities), symptoms and signs (e.g., altered mental status, tachypnea), and laboratory findings.¹³⁶ Most guidelines for treatment of CAP use these or similar measures to divide patients with CAP into 3 levels of severity (e.g., low, moderate, or high severity; patients who can be managed in the outpatient, hospital ward, or ICU settings).

If concomitant influenza infection is not suspected, children with mild-to-moderate CAP who are otherwise

healthy can be treated with amoxicillin, as *S. pneumoniae* is the most common pathogen or a macrolide antibiotic if atypical bacterial pathogens are suspected. IV ampicillin or 3rd generation cephalosporin is recommended for children with suspected pneumococcal pneumonia who are sick enough to be admitted. This regimen will also cover *H. influenzae*, although the latter is necessary if β -lactamase-positive strains are present. Combination therapy with a macrolide is indicated if atypical pathogens are suspected in hospitalized children.¹²⁷

During influenza epidemics and pandemics, *S. aureus* and *S. pyogenes* are additional causes of CAP. Although *S. pyogenes* will be covered by the standard antibiotic regimens for CAP, if influenza is known to be circulating in the community, strong consideration should be made for additional *S. aureus* coverage, particularly in patients who are sick enough to require admission to the intensive care unit, presence of necrotizing/cavitary lesions, or with risk factors for a complicated course (e.g., elderly, comorbidities). Some of the guidelines have made specific recommendations for treating secondary bacterial pneumonias following influenza, given that the frequency of infection by certain bacteria, particularly *S. aureus*, increases in this setting.^{124,125,130,137} During influenza season, in adult patients admitted with CAP, the combination of a beta-lactam antibiotic with fluoroquinolone or macrolide should be sufficient for not only for the common CAP pathogens (e.g., *S. pneumoniae*, *H. influenzae*, atypical bacteria), but also cover MSSA and *S. pyogenes*. However, if MRSA is of concern, vancomycin or linezolid should be started empirically, with the intent to discontinue these antibiotics if culture results return negative.¹²⁴ In children, clindamycin is another alternative for MRSA.¹²⁷

Patients who have symptoms of upper respiratory infection or who do not have evidence of LRTI on chest x-ray (CXR) may be treated symptomatically, with instructions to return if symptoms worsen.

Role of antiviral therapies

Many of the guidelines emphasize that influenza and other respiratory viruses are frequent causes of LRTIs, particularly among children under age 5.¹²⁷ Given the difficulty in distinguishing LRTIs caused by viral, bacterial, or mixed infections, antiviral therapy should be initiated as soon as possible in patients presenting with influenza-like illness or LRTIs during influenza season, prior to receiving confirmatory test results.¹²⁷ Currently, the two main classes of influenza antiviral drugs are the adamantines, which include amantadine and rimantadine, and neuraminidase inhibitors, of which oseltamivir and zanamivir are the only agents that are more widely available. The widespread of adamantine-resistant influenza strains, including the 2009 H1N1 pandemic influenza, has severely limited the utility of this group of antivirals.^{138,139} For patients with milder cases of influ-

enza-like illness and who are otherwise healthy, treatment with oseltamivir or zanamivir is recommended only if started within 48 hours of symptom onset as it can shorten duration of symptoms. In patients admitted to the hospital, however, antiviral treatment with oseltamivir beyond 48 hours after symptom onset may still be beneficial.¹⁴⁰ Newer neuraminidase inhibitors are presently in development or undergoing clinical trials. Peramivir, which is administered intravenously, was made available in the United States during the 2009 H1N1 influenza pandemic under an emergency use authorization. Although it is in phase III trials in the United States, it is approved for use in Japan and Korea.

In theory, treatment with neuraminidase inhibitors might confer some degree of protection against secondary bacterial infections. Studies from animal models suggest that influenza neuraminidase activity contributes to the development of bacterial pneumonia and that neuraminidase inhibitors reduce the susceptibility of influenza-infected animals to secondary bacterial pneumonias.^{27,141} In patients presenting with influenza-like symptoms when influenza is known to be circulating, early treatment with neuraminidase inhibitors (NAI) may prevent lower respiratory tract complications of influenza, although the microbiologic data are not definitive.¹⁴² Clinical trials of NAIs (zanamivir and oseltamivir) suggested that patients with influenza who were treated with NAIs had decreased use of antibiotics for infectious complications, but mainly for bronchitis.^{143–147} In children with influenza infection, oseltamivir treatment reduced the number of prescriptions for antibiotics.¹⁴⁸ Observational studies indicate that timely oseltamivir treatment can reduce the likelihood of pneumonia development.^{149,150} Although shown in animal models of sequential infection,¹⁴¹ it is unclear the degree to which NAIs decrease risk of bacterial pneumonias in influenza-infected humans.

Corticosteroids

Excessive inflammation is believed to contribute to the severity of viral–bacterial co-infections. Hence, interest has arisen in determining whether steroids can mitigate the severity of lung injury from influenza infections. Data from the 2009 H1N1 pandemic on the use of corticosteroids for the treatment of ARDS failed to show any benefit, and perhaps worsened outcomes, including death and infectious complications.^{151,152} At present, corticosteroids are not recommended routinely for post-influenza–bacterial pneumonia although studies are underway to determine their clinical utility in patients with severe bacterial pneumonia.

Concluding remarks

Considerable advances in medical care have occurred over the past century, markedly diminishing the odds of another 1918 influenza pandemic, when bacterial co-infections

appeared to be the predominant cause of death. Vaccinations, antibiotics, and improvements in diagnosis and supportive care all have contributed to improved outcomes from viral-bacterial co-infections. Nonetheless, influenza and pneumonias remain the leading cause of death from infectious disease worldwide, with bacterial pneumonias still contributing to a substantial proportion of deaths during seasonal and pandemic influenza outbreaks. During influenza season, patients presenting with severe LRTIs should be treated empirically with both antiviral and antibacterial agents while awaiting results of microbiologic testing since distinguishing between single and polymicrobial infections can be problematic. An improved understanding of the pathogenetic mechanisms responsible for viral-bacterial co-infections will enable clinicians and public health officials to identify which patients are at risk of developing this potentially fatal complication, and aid in the development of therapeutic approaches aimed at mitigating the severity of co-infections.

Acknowledgements

This work was supported by: NIH R01HL108949 to J.C.D. and NIH/National Center for Advancing Translational Science (NCATS) UCLA CTSI Grant Number UL1TR000124.

Conflicts of interest

Competing interests: J.C.D. has no competing interests.

References

- 1 WHO The top 10 causes of death.
- 2 Murphy SL, Xu J, Kochanek KD (2013) Deaths: Final Data for 2010. National Vital Statistics Reports 61.
- 3 Webb SA, Pettila V, Seppelt I *et al.* Critical care services and 2009 H1N1 influenza in Australia and New Zealand. *N Engl J Med* 2009; 361:1925–1934.
- 4 Gill JR, Sheng ZM, Ely SF *et al.* Pulmonary pathologic findings of fatal 2009 pandemic influenza A/H1N1 viral infections. *Arch Pathol Lab Med* 2010; 134:235–243.
- 5 Lee EH, Wu C, Lee EU *et al.* Fatalities associated with the 2009 H1N1 influenza A virus in New York city. *Clin Infect Dis* 2010; 50:1498–1504.
- 6 Mauad T, Hajjar LA, Callegari GD *et al.* Lung pathology in fatal novel human influenza A (H1N1) infection. *Am J Respir Crit Care Med* 2010; 181:72–79.
- 7 Rice TW, Rubinson L, Uyeki TM *et al.* Critical illness from 2009 pandemic influenza A virus and bacterial coinfection in the United States. *Crit Care Med* 2012; 40:1487–1498.
- 8 Henderson FW, Collier AM, Sanyal MA *et al.* A longitudinal study of respiratory viruses and bacteria in the etiology of acute otitis media with effusion. *N Engl J Med* 1982; 306:1377–1383.
- 9 Ruuskanen O, Arola M, Putto-Laurila A *et al.* Acute otitis media and respiratory virus infections. *Pediatr Infect Dis J* 1989; 8:94–99.
- 10 Bulut Y, Guven M, Otlu B *et al.* Acute otitis media and respiratory viruses. *Eur J Pediatr* 2007; 166:223–228.
- 11 Chonmaitree T, Revai K, Grady JJ *et al.* Viral upper respiratory tract infection and otitis media complication in young children. *Clin Infect Dis* 2008; 46:815–823.
- 12 Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, Andersson B. Cloning of a human parvovirus by molecular screening of respiratory tract samples. *Proc Natl Acad Sci USA* 2005; 102:12891–12896.
- 13 Pettigrew MM, Gent JF, Pyles RB, Miller AL, Nokso-Koivisto J, Chonmaitree T. Viral-bacterial interactions and risk of acute otitis media complicating upper respiratory tract infection. *J Clin Microbiol* 2011; 49:3750–3755.
- 14 Bakaletz LO. Immunopathogenesis of polymicrobial otitis media. *J Leukoc Biol* 2010; 87:213–222.
- 15 Pitkaranta A, Jero J, Arruda E, Virolainen A, Hayden FG. Polymerase chain reaction-based detection of rhinovirus, respiratory syncytial virus, and coronavirus in otitis media with effusion. *J Pediatr* 1998; 133:390–394.
- 16 Pitkaranta A, Roivainen M, Blomgren K *et al.* Presence of viral and bacterial pathogens in the nasopharynx of otitis-prone children. A prospective study. *Int J Pediatr Otorhinolaryngol* 2006; 70:647–654.
- 17 Glezen P, Denny FW. Epidemiology of acute lower respiratory disease in children. *N Engl J Med* 1973; 288:498–505.
- 18 Murphy TF, Henderson FW, Clyde WA Jr, Collier AM, Denny FW. Pneumonia: an eleven-year study in a pediatric practice. *Am J Epidemiol* 1981; 113:12–21.
- 19 Morens DM, Taubenberger JK, Fauci AS. Predominant role of bacterial pneumonia as a cause of death in pandemic influenza: implications for pandemic influenza preparedness. *J Infect Dis* 2008; 198:962–970.
- 20 Stuart-Harris CH, Laird J, Tyrrell DA, Kelsall MH, Pownall M. The relationship between influenza and pneumonia. *J Hyg* 1949; 47:434–448.
- 21 Oswald NC, Shooter RA, Curwen MP. Pneumonia complicating Asian influenza. *Br Med J* 1958; 2:1305–1311.
- 22 Tyrrell DA. The pulmonary complications of influenza as seen in Sheffield in 1949. *Q J Med* 1952; 21:291–306.
- 23 Louri DB, Blumenfeld HL, Ellis JT, Kilbourne ED, Rogers DE. Studies on influenza in the pandemic of 1957–1958. II. Pulmonary complications of influenza. *J Clin Invest* 1959; 38:213–265.
- 24 Bisno AL, Griffin JP, Van Epps KA, Niell HB, Rytel MW. Pneumonia and Hong Kong influenza: a prospective study of the 1968–1969 epidemic. *Am J Med Sci* 1971; 261:251–263.
- 25 McCullers JA, Bartmess KC. Role of neuraminidase in lethal synergism between influenza virus and *Streptococcus pneumoniae*. *J Infect Dis* 2003; 187:1000–1009.
- 26 Peltola VT, McCullers JA. Respiratory viruses predisposing to bacterial infections: role of neuraminidase. *Pediatr Infect Dis J* 2004; 23:S87–S97.
- 27 Peltola VT, Murti KG, McCullers JA. Influenza virus neuraminidase contributes to secondary bacterial pneumonia. *J Infect Dis* 2005; 192:249–257.
- 28 McCullers JA. Insights into the interaction between influenza virus and pneumococcus. *Clin Microbiol Rev* 2006; 19:571–582.
- 29 Zhou H, Haber M, Ray S, Farley MM, Panozzo CA, Klugman KP. Invasive pneumococcal pneumonia and respiratory virus co-infections. *Emerg Infect Dis* 2012; 18:294–297.
- 30 McCullers JA. Preventing and treating secondary bacterial infections with antiviral agents. *Antivir Ther* 2011; 16:123–135.
- 31 Louie JK, Acosta M, Winter K *et al.* (2009) Factors associated with death or hospitalization due to pandemic 2009 influenza A(H1N1) infection in California. *JAMA* 302: 1896–1902.

- 32 Cilloniz C, Ewig S, Menendez R *et al*. Bacterial co-infection with H1N1 infection in patients admitted with community acquired pneumonia. *J Infect* 2012; 65:223–230.
- 33 Martin-Loeches I, Sanchez-Corral A, Diaz E *et al*. Community-acquired respiratory coinfection in critically ill patients with pandemic 2009 influenza A(H1N1) virus. *Chest* 2011; 139:555–562.
- 34 Randolph AG, Vaughn F, Sullivan R *et al*. Critically ill children during the 2009–2010 influenza pandemic in the United States. *Pediatrics* 2011; 128:e1450–e1458.
- 35 Shieh WJ, Blau DM, Denison AM *et al*. 2009 pandemic influenza A (H1N1): pathology and pathogenesis of 100 fatal cases in the United States. *Am J Pathol* 2010; 177:166–175.
- 36 Harms PW, Schmidt LA, Smith LB *et al*. Autopsy findings in eight patients with fatal H1N1 influenza. *Am J Clin Pathol* 2010; 134:27–35.
- 37 Cox CM, Blanton L, Dhara R, Brammer L, Finelli L. 2009 Pandemic influenza A (H1N1) deaths among children—United States, 2009–2010. *Clin Infect Dis* 2011; 52(Suppl 1):S69–S74.
- 38 Weinberger DM, Simonsen L, Jordan R, Steiner C, Miller M, Viboud C. Impact of the 2009 influenza pandemic on pneumococcal pneumonia hospitalizations in the United States. *J Infect Dis* 2012; 205:458–465.
- 39 Nelson GE, Gershman KA, Swerdlow DL, Beall BW, Moore MR. Invasive pneumococcal disease and pandemic (H1N1) 2009, Denver, Colorado, USA. *Emerg Infect Dis* 2012; 18:208–216.
- 40 Fleming-Dutra KE, Taylor T, Link-Gelles R *et al*. Effect of the 2009 influenza A(H1N1) pandemic on invasive pneumococcal pneumonia. *J Infect Dis* 2013; 207:1135–1143.
- 41 Ampofo K, Bender J, Sheng X *et al*. Seasonal invasive pneumococcal disease in children: role of preceding respiratory viral infection. *Pediatrics* 2008; 122:229–237.
- 42 Kim PE, Musher DM, Glezen WP, Rodriguez-Barradas MC, Nahm WK, Wright CE. Association of invasive pneumococcal disease with season, atmospheric conditions, air pollution, and the isolation of respiratory viruses. *Clin Infect Dis* 1996; 22:100–106.
- 43 Talbot TR, Poehling KA, Hartert TV *et al*. Seasonality of invasive pneumococcal disease: temporal relation to documented influenza and respiratory syncytial viral circulation. *Am J Med* 2005; 118:285–291.
- 44 Murdoch DR, Jennings LC. Association of respiratory virus activity and environmental factors with the incidence of invasive pneumococcal disease. *J Infect* 2009; 58:37–46.
- 45 Honkinen M, Lahti E, Osterback R, Ruuskanen O, Waris M. Viruses and bacteria in sputum samples of children with community-acquired pneumonia. *Clin Microbiol Infect* 2012; 18:300–307.
- 46 Korppi M. Mixed viral-bacterial pulmonary infections in children. *Pediatr Pulmonol Suppl* 1999; 18:110–112.
- 47 Michelow IC, Olsen K, Lozano J *et al*. Epidemiology and clinical characteristics of community-acquired pneumonia in hospitalized children. *Pediatrics* 2004; 113:701–707.
- 48 Tsoia MN, Psarras S, Bossios A *et al*. Etiology of community-acquired pneumonia in hospitalized school-age children: evidence for high prevalence of viral infections. *Clin Infect Dis* 2004; 39:681–686.
- 49 Rudan I, O'Brien KL, Nair H *et al*. Epidemiology and etiology of childhood pneumonia in 2010: estimates of incidence, severe morbidity, mortality, underlying risk factors and causative pathogens for 192 countries. *J Glob Health* 2013; 3:10401.
- 50 Jennings LC, Anderson TP, Beynon KA *et al*. Incidence and characteristics of viral community-acquired pneumonia in adults. *Thorax* 2008; 63:42–48.
- 51 Johnstone J, Majumdar SR, Fox JD, Marrie TJ. Viral infection in adults hospitalized with community-acquired pneumonia: prevalence, pathogens, and presentation. *Chest* 2008; 134:1141–1148.
- 52 McCullers JA, Reh JE. Lethal synergism between influenza virus and *Streptococcus pneumoniae*: characterization of a mouse model and the role of platelet-activating factor receptor. *J Infect Dis* 2002; 186:341–350.
- 53 Sun K, Metzger DW. Inhibition of pulmonary antibacterial defense by interferon-gamma during recovery from influenza infection. *Nat Med* 2008; 14:558–564.
- 54 Shahangian A, Chow EK, Tian X *et al*. Type I IFNs mediate development of postinfluenza bacterial pneumonia in mice. *J Clin Invest* 2009; 119:1910–1920.
- 55 Ballinger MN, Standiford TJ. Postinfluenza bacterial pneumonia: host defenses gone awry. *J Interferon Cytokine Res* 2010; 30:643–652.
- 56 Carlson JL, Collier AM, Hu SS. Acquired ciliary defects in nasal epithelium of children with acute viral upper respiratory infections. *N Engl J Med* 1985; 312:463–468.
- 57 Pittet LA, Hall-Stoodley L, Rutkowski MR, Harmsen AG. Influenza virus infection decreases tracheal mucociliary velocity and clearance of *Streptococcus pneumoniae*. *Am J Respir Cell Mol Biol* 2010; 42:450–460.
- 58 Selinger DS, Reed WP, McLaren LC. Model for studying bacterial adherence to epithelial cells infected with viruses. *Infect Immun* 1981; 32:941–944.
- 59 Plotkowski MC, Puchelle E, Beck G, Jacquot J, Hannoun C. Adherence of type I *Streptococcus pneumoniae* to tracheal epithelium of mice infected with influenza A/PR8 virus. *Am Rev Respir Dis* 1986; 134:1040–1044.
- 60 Avadhanula V, Rodriguez CA, Devincenzo JP *et al*. Respiratory viruses augment the adhesion of bacterial pathogens to respiratory epithelium in a viral species- and cell type-dependent manner. *J Virol* 2006; 80:1629–1636.
- 61 van der Sluijs KF, van Elden LJ, Nijhuis M *et al*. Involvement of the platelet-activating factor receptor in host defense against *Streptococcus pneumoniae* during postinfluenza pneumonia. *Am J Physiol Lung Cell Mol Physiol* 2006; 290:L194–L199.
- 62 Diavatopoulos DA, Short KR, Price JT *et al*. Influenza A virus facilitates *Streptococcus pneumoniae* transmission and disease. *FASEB J* 2010; 24:1789–1798.
- 63 Nakamura S, Davis KM, Weiser JN. Synergistic stimulation of type I interferons during influenza virus coinfection promotes *Streptococcus pneumoniae* colonization in mice. *J Clin Invest* 2011; 121:3657–3665.
- 64 Short KR, Reading PC, Wang N, Diavatopoulos DA, Wijburg OL. Increased nasopharyngeal bacterial titers and local inflammation facilitate transmission of *Streptococcus pneumoniae*. *mBio [Research Support, Non-U.S. Gov't]* 2012; 3:e00255–12.
- 65 McCullers JA, McAuley JL, Browall S *et al*. Influenza enhances susceptibility to natural acquisition of and disease due to *Streptococcus pneumoniae* in ferrets. *J Infect Dis* 2010; 202:1287–1295.
- 66 Kleinerman ES, Daniels CA, Polisson RP, Snyderman R. Effect of virus infection on the inflammatory response. Depression of macrophage accumulation in influenza-infected mice. *Am J Pathol* 1976; 85:373–382.
- 67 Kleinerman ES, Snyderman R, Daniels CA. Depressed monocyte chemotaxis during acute influenza infection. *Lancet* 1975; 2:1063–1066.
- 68 Kleinerman ES, Snyderman R, Daniels CA. Depression of human monocyte chemotaxis by herpes simplex and influenza viruses. *J Immunol* 1974; 113:1562–1567.
- 69 Astry CL, Jakab GJ. Influenza virus-induced immune complexes suppress alveolar macrophage phagocytosis. *J Virol* 1984; 50:287–292.
- 70 Jakab GJ. Immune impairment of alveolar macrophage phagocytosis during influenza virus pneumonia. *Am Rev Respir Dis* 1982; 126:778–782.

- 71 Jakab GJ, Warr GA, Knight ME. Pulmonary and systemic defenses against challenge with *Staphylococcus aureus* in mice with pneumonia due to influenza A virus. *J Infect Dis* 1979; 140:105–108.
- 72 Nickerson CL, Jakab GJ. Pulmonary antibacterial defenses during mild and severe influenza virus infection. *Infect Immun* 1990; 58:2809–2814.
- 73 Raza MW, Blackwell CC, Elton RA, Weir DM. Bactericidal activity of a monocytic cell line (THP-1) against common respiratory tract bacterial pathogens is depressed after infection with respiratory syncytial virus. *J Med Microbiol* 2000; 49:227–233.
- 74 Craft AW, Reid MM, Low WT. Effect of virus infections on polymorph function in children. *Br Med J* 1976; 1:1570.
- 75 Ruutu P. Depression of rat neutrophil exudation and motility by influenza virus. *Scand J Immunol* 1977; 6:1113–1120.
- 76 Ruutu P, Vaheeri A, Kosunen TU. Depression of human neutrophil motility by influenza virus in vitro. *Scand J Immunol* 1977; 6:897–906.
- 77 Schlesinger JJ, Ernst C, Weinstein L. Letter: inhibition of human neutrophil chemotaxis by influenza virus. *Lancet* 1976; 1:650–651.
- 78 Larson HE, Parry RP, Tyrrell DA. Impaired polymorphonuclear leucocyte chemotaxis after influenza virus infection. *British J Dis Chest* 1980; 74:56–62.
- 79 Martin RR, Couch RB, Greenberg SB, Cate TR, Warr GA. Effects of infection with influenza virus on the function of polymorphonuclear leukocytes. *J Infect Dis* 1981; 144:279–280.
- 80 Larson HE, Blades R. Impairment of human polymorphonuclear leucocyte function by influenza virus. *Lancet* 1976; 1:283.
- 81 Abramson JS, Mills EL. Depression of neutrophil function induced by viruses and its role in secondary microbial infections. *Rev Infect Dis* 1988; 10:326–341.
- 82 Didierlaurent A, Goulding J, Patel S *et al*. Sustained desensitization to bacterial Toll-like receptor ligands after resolution of respiratory influenza infection. *J Exp Med* 2008; 205:323–329.
- 83 Kudva A, Scheller EV, Robinson KM *et al*. Influenza A inhibits Th17-mediated host defense against bacterial pneumonia in mice. *J Immunol* 2011; 186:1666–1674.
- 84 Li W, Moltedo B, Moran TM. Type I interferon induction during influenza virus infection increases susceptibility to secondary *Streptococcus pneumoniae* infection by negative regulation of gamma-delta T cells. *J Virol* 2012; 86:12304–12312.
- 85 Tian X, Xu F, Lung WY *et al*. Poly I:C enhances susceptibility to secondary pulmonary infections by gram-positive bacteria. *PLoS ONE* 2012; 7:e41879.
- 86 de Jong MD, Simmons CP, Thanh TT *et al*. Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. *Nat Med* 2006; 12:1203–1207.
- 87 Kobasa D, Takada A, Shinya K *et al*. Enhanced virulence of influenza A viruses with the haemagglutinin of the 1918 pandemic virus. *Nature* 2004; 431:703–707.
- 88 Zheng BJ, Chan KW, Lin YP *et al*. Delayed antiviral plus immunomodulator treatment still reduces mortality in mice infected by high inoculum of influenza A/H5N1 virus. *Proc Natl Acad Sci USA* 2008; 105:8091–8096.
- 89 Seki M, Yanagihara K, Higashiyama Y *et al*. Immunokinetics in severe pneumonia due to influenza virus and bacteria coinfection in mice. *Eur Respir J* 2004; 24:143–149.
- 90 Zavitz CC, Bauer CM, Gaschler GJ *et al*. Dysregulated macrophage-inflammatory protein-2 expression drives illness in bacterial superinfection of influenza. *J Immunol* 2010; 184:2001–2013.
- 91 Simmons C, Farrar J. Insights into inflammation and influenza. *N Engl J Med* 2008; 359:1621–1623.
- 92 Sun J, Madan R, Karp CL, Braciale TJ. Effector T cells control lung inflammation during acute influenza virus infection by producing IL-10. *Nat Med* 2009; 15:277–284.
- 93 van der Sluijs KF, Nijhuis M, Levels JH *et al*. Influenza-induced expression of indoleamine 2,3-dioxygenase enhances interleukin-10 production and bacterial outgrowth during secondary pneumococcal pneumonia. *J Infect Dis* 2006; 193:214–222.
- 94 van der Sluijs KF, van Elden LJ, Nijhuis M *et al*. IL-10 is an important mediator of the enhanced susceptibility to pneumococcal pneumonia after influenza infection. *J Immunol* 2004; 172:7603–7609.
- 95 WHO (2012) Global routine vaccination coverage, 2011. Releve epidemiologique hebdomadaire/Section d'hygiene du Secretariat de la Societe des Nations = Weekly epidemiological record/Health Section of the Secretariat of the League of Nations; 87: 432–435.
- 96 Madhi SA, Ludewick H, Kuwanda L *et al*. Pneumococcal coinfection with human metapneumovirus. *J Infect Dis* 2006; 193:1236–1243.
- 97 Simonsen L, Taylor RJ, Young-Xu Y *et al*. Impact of pneumococcal conjugate vaccination of infants on pneumonia and influenza hospitalization and mortality in all age groups in the United States. *mBio* 2011; 2:e00309–e00310.
- 98 Dominguez A, Castilla Catalan J, Godoy P *et al*. Effectiveness of vaccination with 23-valent pneumococcal polysaccharide vaccine in preventing hospitalization with laboratory confirmed influenza during the 2009–2010 and 2010–2011 seasons. *Hum Vaccin Immunother* 2013; 9:330–334.
- 99 Dominguez A, Castilla J, Godoy P *et al*. Benefit of conjugate pneumococcal vaccination in preventing influenza hospitalization in children: a case-control study. *Pediatr Infect Dis J* 2013; 32:330–334.
- 100 Govaert TM, Thijs CT, Masurel N *et al*. The efficacy of influenza vaccination in elderly individuals. A randomized double-blind placebo-controlled trial. *JAMA* 1994; 272:1661–1665.
- 101 Nichol KL, Nordin JD, Nelson DB, Mullooly JP, Hak E. Effectiveness of influenza vaccine in the community-dwelling elderly. *N Engl J Med* 2007; 357:1373–1381.
- 102 Clements DA, Langdon L, Bland C, Walter E. Influenza A vaccine decreases the incidence of otitis media in 6- to 30-month-old children in day care. *Arch Pediatr Adolesc Med* 1995; 149:1113–1117.
- 103 Ohmit SE, Monto AS. Influenza vaccine effectiveness in preventing hospitalization among the elderly during influenza type A and type B seasons. *Int J Epidemiol* 1995; 24:1240–1248.
- 104 Ozgur SK, Beyazova U, Kemaloglu YK *et al*. Effectiveness of inactivated influenza vaccine for prevention of otitis media in children. *Pediatr Infect Dis J* 2006; 25:401–404.
- 105 Foster DA, Talsma A, Furumoto-Dawson A *et al*. Influenza vaccine effectiveness in preventing hospitalization for pneumonia in the elderly. *Am J Epidemiol* 1992; 136:296–307.
- 106 Miller WT Jr, Mickus TJ, Barbosa E Jr *et al*. CT of viral lower respiratory tract infections in adults: comparison among viral organisms and between viral and bacterial infections. *AJR Am J Roentgenol* 2011; 197:1088–1095.
- 107 Flanders SA, Stein J, Shochat G *et al*. Performance of a bedside C-reactive protein test in the diagnosis of community-acquired pneumonia in adults with acute cough. *Am J Med* 2004; 116:529–535.
- 108 Schuetz P, Amin DN, Greenwald JL. Role of procalcitonin in managing adult patients with respiratory tract infections. *Chest* 2012; 141:1063–1073.
- 109 Gibot S, Cravoisy A, Levy B, Bene MC, Faure G, Bollaert PE. Soluble triggering receptor expressed on myeloid cells and the diagnosis of pneumonia. *N Engl J Med* 2004; 350:451–458.
- 110 Muller B, Gencay MM, Gibot S *et al*. Circulating levels of soluble triggering receptor expressed on myeloid cells (sTREM)-1 in community-acquired pneumonia. *Crit Care Med* 2007; 35:990–991.
- 111 Cuquemelle E, Soulis F, Villers D *et al*. Can procalcitonin help identify associated bacterial infection in patients with severe

- influenza pneumonia? A multicentre study. *Intensive Care Med* 2011; 37:796–800.
- 112 Bjerrum L, Gahrn-Hansen B, Munck AP. C-reactive protein measurement in general practice may lead to lower antibiotic prescribing for sinusitis. *Br J Gen Pract* 2004; 54:659–662.
- 113 Cals JW, Butler CC, Hopstaken RM, Hood K, Dinant GJ. Effect of point of care testing for C reactive protein and training in communication skills on antibiotic use in lower respiratory tract infections: cluster randomised trial. *BMJ* 2009; 338:b1374.
- 114 Cals JW, Schot MJ, de Jong SA, Dinant GJ, Hopstaken RM. Point-of-care C-reactive protein testing and antibiotic prescribing for respiratory tract infections: a randomized controlled trial. *Annals of family medicine* 2010; 8:124–133.
- 115 Christ-Crain M, Jaccard-Stolz D, Bingisser R *et al.* Effect of procalcitonin-guided treatment on antibiotic use and outcome in lower respiratory tract infections: cluster-randomised, single-blinded intervention trial. *Lancet* 2004; 363:600–607.
- 116 Stolz D, Christ-Crain M, Bingisser R *et al.* Antibiotic treatment of exacerbations of COPD: a randomized, controlled trial comparing procalcitonin-guidance with standard therapy. *Chest* 2007; 131:9–19.
- 117 Schuetz P, Christ-Crain M, Thomann R *et al.* (2009) Effect of procalcitonin-based guidelines vs standard guidelines on antibiotic use in lower respiratory tract infections: the ProHOSP randomized controlled trial. *JAMA* 302: 1059–1066.
- 118 Burkhardt O, Ewig S, Haagen U *et al.* Procalcitonin guidance and reduction of antibiotic use in acute respiratory tract infection. *Eur Respir J* 2010; 36:601–607.
- 119 Haran JP, Beaudoin FL, Suner S, Lu S. C-reactive protein as predictor of bacterial infection among patients with an influenza-like illness. *Am J Emerg Med* 2013; 31:137–144.
- 120 Diez-Padriza N, Bassat Q, Machevo S *et al.* Procalcitonin and C-reactive protein for invasive bacterial pneumonia diagnosis among children in Mozambique, a malaria-endemic area. *PLoS ONE* 2010; 5:e13226.
- 121 (ALAT) GdtLALdT (2004) Update to the Latin American Thoracic Association (ALAT) recommendations on community acquired pneumonia. *Arch Bronconeumol* 40: 364–374.
- 122 Japanese Respiratory Society (2006) Guidelines for the management of community acquired pneumonia in adults, revised edition. *Respirology* 11 Suppl 3: S79–S133.
- 123 South African Thoracic Society Working Group (2007) Management of community-acquired pneumonia in adults. *South African Med J = Suid-Afrikaanse tydskrif vir geneeskunde* 97: 1296–1306.
- 124 Mandell LA, Wunderink RG, Anzueto A *et al.* Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. *Clin Infect Dis* 2007; 44(Suppl 2):S27–S72.
- 125 Lim WS, Baudouin SV, George RC *et al.* (2009) BTS guidelines for the management of community acquired pneumonia in adults: update 2009. *Thorax* 64 Suppl 3: iii1–iii55.
- 126 Correa Rde A, Lundgren FL, Pereira-Silva JL *et al.* Brazilian guidelines for community-acquired pneumonia in immunocompetent adults - 2009. *J Bras Pneumol* 2009; 35:574–601.
- 127 Bradley JS, Byington CL, Shah SS *et al.* The management of community-acquired pneumonia in infants and children older than 3 months of age: clinical practice guidelines by the Pediatric Infectious Diseases Society and the Infectious Diseases Society of America. *Clin Infect Dis* 2011; 53:e25–e76.
- 128 Gupta D, Agarwal R, Aggarwal AN *et al.* Guidelines for diagnosis and management of community- and hospital-acquired pneumonia in adults: joint ICS/NCCP(I) recommendations. *Lung India* 2012; 29: S27–S62.
- 129 Mandell LA. Severe community-acquired pneumonia (CAP) and the Infectious Diseases Society of America/American Thoracic Society CAP guidelines prediction rule: validated or not. *Clin Infect Dis* 2009; 48:386–388.
- 130 Spindler C, Stralin K, Eriksson L *et al.* Swedish guidelines on the management of community-acquired pneumonia in immunocompetent adults—Swedish Society of Infectious Diseases 2012. *Scand J Infect Dis* 2012; 44:885–902.
- 131 Almirall J, Bolibar I, Vidal J *et al.* Epidemiology of community-acquired pneumonia in adults: a population-based study. *Eur Respir J* 2000; 15:757–763.
- 132 Wiemken TL, Peyrani P, Ramirez JA. Global changes in the epidemiology of community-acquired pneumonia. *Semin Respir Crit Care Med* 2012; 33:213–219.
- 133 Zar HJ. Pneumonia in HIV-infected and HIV-uninfected children in developing countries: epidemiology, clinical features, and management. *Curr Opin Pulm Med* 2004; 10:176–182.
- 134 CDC (2003) Drug-Resistant *Streptococcus pneumoniae* (DRSP) Surveillance Manual.
- 135 Network EARS. Available at <http://ecdc.europa.eu/en/activities/surveillance/Pages/index.aspx>
- 136 Aujesky D, Auble TE, Yealy DM *et al.* Prospective comparison of three validated prediction rules for prognosis in community-acquired pneumonia. *Am J Med* 2005; 118:384–392.
- 137 Woodhead M, Blasi F, Ewig S *et al.* Guidelines for the management of adult lower respiratory tract infections—full version. *Clin Microbiol Infect: Offic Pub Europ Soci Clin Microbiol Infect Dis* 2011; 17(Suppl 6):E1–E59.
- 138 Bright RA, Medina MJ, Xu X *et al.* Incidence of adamantane resistance among influenza A (H3N2) viruses isolated worldwide from 1994 to 2005: a cause for concern. *Lancet* 2005; 366:1175–1181.
- 139 Leang SK, Deng YM, Shaw R *et al.* Influenza antiviral resistance in the Asia-Pacific region during 2011. *Antiviral Res* 2013; 97:206–210.
- 140 Viasus D, Pano-Pardo JR, Pachon J *et al.* Timing of oseltamivir administration and outcomes in hospitalized adults with pandemic 2009 influenza A(H1N1) virus infection. *Chest* 2011; 140:1025–1032.
- 141 McCullers JA. Effect of antiviral treatment on the outcome of secondary bacterial pneumonia after influenza. *J Infect Dis* 2004; 190:519–526.
- 142 Hernan MA, Lipsitch M. Oseltamivir and risk of lower respiratory tract complications in patients with flu symptoms: a meta-analysis of eleven randomized clinical trials. *Clin Infect Dis* 2011; 53:277–279.
- 143 Hayden FG, Osterhaus AD, Treanor JJ *et al.* Efficacy and safety of the neuraminidase inhibitor zanamivir in the treatment of influenza-zavirus infections. GG167 Influenza Study Group. *N Engl J Med* 1997; 337:874–880.
- 144 Kaiser L, Keene ON, Hammond JM, Elliott M, Hayden FG. Impact of zanamivir on antibiotic use for respiratory events following acute influenza in adolescents and adults. *Arch Intern Med* 2000; 160:3234–3240.
- 145 Kaiser L, Wat C, Mills T, Mahoney P, Ward P, Hayden F. Impact of oseltamivir treatment on influenza-related lower respiratory tract complications and hospitalizations. *Arch Intern Med* 2003; 163:1667–1672.
- 146 Nicholson KG, Aoki FY, Osterhaus AD *et al.* Efficacy and safety of oseltamivir in treatment of acute influenza: a randomised controlled trial. Neuraminidase Inhibitor Flu Treatment Investigator Group. *Lancet* 2000; 355:1845–1850.
- 147 Treanor JJ, Hayden FG, Vrooman PS *et al.* Efficacy and safety of the oral neuraminidase inhibitor oseltamivir in treating acute influenza: a randomized controlled trial. US Oral Neuraminidase Study Group. *JAMA* 2000; 283: 1016–1024.

- 148** Whitley RJ, Hayden FG, Reisinger KS *et al.* Oral oseltamivir treatment of influenza in children. *Pediatr Infect Dis J* 2001; 20:127–133.
- 149** Hsu J, Santesso N, Mustafa R *et al.* Antivirals for treatment of influenza: a systematic review and meta-analysis of observational studies. *Ann Intern Med* 2012; 156:512–524.
- 150** Muthuri SG, Myles PR, Venkatesan S, Leonardi-Bee J, Nguyen-Van-Tam JS. Impact of neuraminidase inhibitor treatment on outcomes of public health importance during the 2009-2010 influenza A (H1N1) pandemic: a systematic review and meta-analysis in hospitalized patients. *J Infect Dis* 2013; 207:553–563.
- 151** Kim SH, Hong SB, Yun SC *et al.* Corticosteroid treatment in critically ill patients with pandemic influenza A/H1N1 2009 infection: analytic strategy using propensity scores. *Am J Respir Crit Care Med* 2011; 183:1207–1214.
- 152** Brun-Buisson C, Richard JC, Mercat A, Thiebaut AC, Brochard L. Early corticosteroids in severe influenza A/H1N1 pneumonia and acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2011; 183:1200–1206.