REVIEW

The dangerous synergism between Influenza and *Streptococcus pneumoniae* and innovative perspectives of vaccine prevention

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Key words

Synergism • Influenza • *Streptococcus pneumoniae* • Pneumococcal conjugate vaccines

Summary

Secondary bacterial pneumonia, particularly sustained by Streptococcus pneumoniae (Sp), represents an important cause of excess mortality during both influenza epidemics and pandemics. The lethal synergism between influenza virus and Sp was first suggested by studies performed on samples collected during autopsy from victims of 1918 influenza pandemic, and recently confirmed by data collected during the 2009 A/H1N1v influenza pandemic. Moreover, researches carried out in animal model contributed to partially clarify the pathogenic mechanisms underlying the synergism between these two etiological agents.
Since 2000, a seven-valent pneumococcal conjugate vaccine (PCV7) was introduced in the US, and in the following year in Europe, determining substantial and almost immediate benefits in terms of reduction of invasive pneumococcal disease (IPD) in both vaccinated children and adults through induction of herd protection. Furthermore, several researches have recently demonstrated the capacity of the PCV7 to prevent community-acquired pneumonia (CAP) and, in particular, influenza-associated pneumonia hospitalisations among children. Taking into account the above-mentioned positive results obtained with PCV7, the availability of a new generation of conjugate pneumococcal vaccine with an enlarged antigenic spectrum (i.e. PCV13) offers promising perspectives, to improve the control of influenza through the protection offered against its major complications, particularly CAP, not only in children, but also among adults.

Introduction

Annual influenza epidemics globally cause significant morbidity and mortality [1]. High rates of influenza hospitalisations occur in healthy infants and young children as well as in adults with risk factors, whereas most deaths occur in the elderly [2-4]. In addition to annual epidemics, influenza virus causes occasional pandemics, which may have an extremely high burden worldwide. Secondary bacterial pneumonia represents an important cause of excess mortality during both influenza epidemics and pandemics [5].
The lethal association between influenza and bacterial infections was first hypothesized from 1890 to 1950, when most observers believed fatal influenza to be a polymicrobial infection in which an inciting agent of low pathogenicity, most of which have now been identified as viruses, acted synergistically with known pneumopathogenic bacteria [5]. This synergism came into particular focus during the influenza pandemic of 1918, when 40 to 50 million deaths occurred, many because of secondary bacterial pneumonia. In particular, recent studies, examining recut tissue specimens and reviewing epidemiologic, pathologic, and microbiologic data, have highlighted the major role of *Streptococcus pneumoniae* (*Sp*) in determining excess mortality during the 1918 influenza pandemic [5, 6]. These findings have been recently confirmed by data collected during the 2009 A/H1N1v influenza pandemic [7-10].
Numerous studies, performed in animal model, demonstrated that influenza A virus infection causes significant changes in the respiratory tract, such as epithelial damage, alterations in airway function, up-regulation and exposure of receptors, and impairment of the innate immune response, thus severely increasing susceptibility to secondary *Sp* infection [11-13]. The most important evidences that have suggested the lethal synergism between influenza virus and *Sp,* both in animal model and in human beings, are discussed below in the text.
Since 2000, a seven-valent pneumococcal conjugate vaccine (PCV7) was introduced in the US, and in the following year in Europe, determining substantial and almost immediate benefits in terms of reduction of invasive pneumococcal disease (IPD) in both vaccinated children and adults through induction of herd protection [14-20]. During the last years, evidences of the positive impact of this vaccine on community-acquired pneumonia (CAP), in terms of reduction in the number of hospitalisations and ambulatory visits for all-cause pneumonia, have been also growing up in the literature [21-24]. This is of particular meaning considering the role of secondary bacterial infection as a major complication of influenza. Furthermore, recent studies have demonstrated a significantly fewer influenza-associated

pneumonia hospitalisations among children immunized with PCV7 [25, 26].
The availability of a new generation of conjugate pneumococcal vaccines with an enlarged antigenic spectrum (i.e. PCV13), together with the positive results obtained with PCV7, as discussed below, offer promising perspectives to reduce the burden of influenza, improving the protection against its major complications, particularly CAP, not only in children, but also among adults.

Influenza and *Streptococcus pneumoniae* sequential infections in animal model

Since seventies a correlation between influenza infection and severity of illness due to streptococcus was reported in animal model.
Squirrel monkeys, inoculated intratracheally with influenza A virus (H3N2), responded with clinical illness including signs such as fever, sneezing or coughing, coryza, and increased respiratory rates. Necropsy studies performed six days after inoculation revealed bronchopneumonia in addition to a mild tracheitis. Squirrel monkeys infected with both influenza virus and *Sp* intratracheally died four to six days later after developing severe illness characterized by fever, bacteraemia, lethargy, anorexia, coughing, laboured breathing, and bronchopneumonia. At necropsy these monkeys had more extensive and severe bronchopneumonia than was seen in monkeys infected with either organism alone [27].
More recently, studies performed in mice have contributed to elucidate the interaction between *Sp* and influenza. Mouse models showed that influenza infection preceding pneumococcal challenge primed for pneumonia and led to 100% mortality. Differently, reversal of the order of administration led to protection from influenza and improved survival. Findings of this study showed that influenza virus infection primes for development of lethal pneumococcal pneumonia with a dose dependent mechanism and that the effect is stronger if exposure to pneumococcal occurs seven days after influenza infection resulting in a more severe pathologic picture than a classic lobar pneumonia, otherwise if pneumococcal administration precedes influenza infection, survivals to this latter is improved. The meaning of this observation has still to be clarified [11].
A number of studies performed in animal model have contributed to partially clarify the pathogenic mechanisms underlying the synergism between influenza virus and *Sp*: the main findings are summarized in Table I.

Influenza and *Streptococcus pneumoniae*: evidence from the past

Although a widespread conviction still exists, also among physicians, that the 1918 influenza had a fulminate course, led to death within hours or days as a result of acute respiratory distress syndrome (ARDS) directly caused by primary viral pneumonia, these events were an unusual characteristic in this pandemic, representing a small proportion of the fatal cases, yet. It has been estimated that less than 5% of deaths occurred within 3 days after the onset of flu symptoms, while most deaths occurred within 7-14 days, and about 30% of deaths occurred more than 14 days after initial symptoms [28].
The examination of recut lung tissue sections, obtained during autopsy from 58 influenza victims, revealed, in virtually all cases, compelling histologic evidence of severe acute bacterial pneumonia, either as the predominant pathology or in conjunction with underlying pathologic features now believed to be associated with influenza virus infection [5]. This study also examined the bacterial culture results of 96 post-mortem lung tissue culture series, which examined 5266 subjects, demonstrating that 92.7% of autopsy lung cultures were positive for ≥ 1 bacterium and that *Sp* was the single most common infecting bacterium isolated [5].
In accordance with these findings, a recent review, which analysed the studies that had investigated more than 10 sterile-site ante-mortem cultures from adults with pneu-

Tab. I. Pathogenic mechanisms underlying the synergism between influenza virus and *Streptococcus pneumonia.*

Epithelial damage	Increased exposure of binding sites for the bacterium (eg, elements of the basal membrane, such as fibrinogen)
Reduced activity in the mechanisms of airway protection	Impaired ciliary function (decreased beat rate, non-coordinated activity) Impaired ciliary function (decreased beat rate, non-coordinated activity) Obstruction of small airways due to the destruction of the surfactant Increase in secretions of mucinous
Up-regulation and increased exposure of specific adhesion receptors of the bacterium	Sialic acid cleavage by the viral NA determines the exposure of receptors at the level of the lower airways that binds Pneumococcus Pro-inflammatory cytokines up-regulate the expression of cellular receptors (eg PAFr) that promote the adhesion of Pneumococcus
Down-regulation of the innate immune response	Increased interferon-γ: down Regulation of scavenger receptor (MARCO) of alveolar macrophages Influenza virus causes apoptosis (in vitro) and altered function (in vivo) of neutrophils Influenza virus impairs the activity of macrophages, reducing chemotaxis and by suppressing phagocytosis

monia and those without pneumonia, showed that bacteria could be identified in only a few blood cultures for patients who had influenza but not pneumonia (mean among all the reports, < 1%), whereas these agents were isolated in 16% of samples collected from patients with influenza-associated pneumonia and in 40% of samples from patients who died. Moreover, 80% of pleural-fluid and lung cultures from patients with pneumonia yielded bacteria, and *Sp* was the most frequent bacterium isolated, comprising 71% of positive cultures [6].
Therefore, bacteria were commonly recovered from human samples of living pneumonia cases and fatal cases in the 1918 pandemic, suggesting that these microbiological agents, particularly *Sp*, played an important role in the pathogenesis of the influenza associated pneumonia and death in this pandemic.

Influenza and *Streptococcus pneumoniae*: evidence from the 2009 A/H1N1V pandemic

In April 2009, novel swine-origin influenza A (H1N1) virus was identified in California and Mexico as a cause of human respiratory disease. On 11st June 2009, the World Health Organization (WHO) declared a pandemic outbreak of respiratory illness associated with this novel influenza virus.
Since in previous influenza pandemics studies of autopsy specimens have demonstrated that most deaths attributed to influenza A virus infection occurred concurrently with bacterial pneumonia, several studies investigated the interaction between these microorganisms in the context of the last pandemic event.
A report by Center for Disease Control and prevention (CDC) analysed samples from 77 US patients with fatal cases of confirmed 2009 pandemic influenza A/H1N1v, evidencing concurrent bacterial infection in 22 (29%) cases of the 77 patients, including 10 (45.5%) isolations of *Sp* [7].
A post mortem study reported the pathological findings from 21 patients with proven novel A/H1N1v infection who died during the winter period of the 2009 pandemic in Sao Paulo, Brazil, evidencing as the cause of death in all patients was extensive involvement of the lungs and founding evidence of bacterial coinfection in 8 (38.1%) out of 21 patients: in 6 (28.6%) patients bacteriological analysis by culture of bronchial aspirate and/or tissue PCR revealed *Sp* as the etiological agent [8].
A study, performed in 337 Argentinian adult patients with confirmed, probable, or suspected cases of 2009 influenza A/H1N1v admitted to 35 Intensive Care Units with acute respiratory failure requiring mechanical ventilation, demonstrated coexistent bacterial pneumonia on admission in 25% of the patients. *Sp* was isolated in 35% of these subjects with bacterial coinfection and this pathogen was found to be associated with worst prognosis, despite concurrent antibiotic treatment on admission [9].
Another Argentinian study examined nasopharyngeal swab samples from 199 cases of mild and severe influenza A/H1N1v infection, identifying at least one additional pathogenic agent importance in 152 (76%) samples. *Sp* was isolated in 62 (40.8%) out of the 152 positive samples and its presence was strongly correlated with severe disease also in this research [10].

Innovative perspectives of vaccine prevention

Influenza virus and *Sp* represent two of the most important pathogens affecting humans today in Western Countries, being public health priorities. The demonstration of their ability to work synergistically further evidences the need for improving strategies for the control of influenza and its most frequent complication, which is secondary bacterial pneumonia.
Primary prevention of both influenza and *Sp* associated diseases is largely based on vaccination. In the last decades, the implementation of innovative strategies to improve the performance of influenza vaccines, namely, the addition of different adjuvants (i.e., MF59- and AS03-vaccines, virosomal formulations), the use of alternative routes of administration or manufacture (i.e., intradermal, nasal and oral vaccines and cell culture- and reverse genetic-based vaccines) or of high doses of antigen, has contributed to substantially improve the protection conferred against the disease [29].
At the same time, the availability of pneumococcal conjugated vaccines has contributed to increase the protection against *Sp* associated diseases. In particular, the wide use of PCV7 in universal childhood vaccination programs, both in US and Europe, has determined a significant and rapid impact on IPD in the target paediatric population: monitoring a sample of the US population of nearly 1.26 million subjects, nearly a 100% and a 76% reduction in IPD rates, as related to vaccine serotypes and to all *Sp*-types, were registered among children aged less than 5 years, comparing the pre- versus post-vaccination periods, with values declining from 81.9/105 to 0.4/105 and from 98.7/105 to 23.6/105, respectively [15]. Similar findings, concerning the effects of the PCV7 against IPD, were also obtained in Europe and Canada, thus further confirming the positive effects of the universal children immunization campaign worldwide [16-20].
Surprisingly, the universal vaccination of newborns rapidly determined an indirect protection against IPD also among the non-vaccinated: notably, in 2003, the vaccine prevented more than twice as many IPD cases in the US, just through this indirect effect [14]. This positive effect persisted during the years following the introduction of the universal immunization program, and resulted particularly marked in subjects aged ≥ 65 years, a group at higher risk for IPD than the young adult population: comparing average data collected in 1998-1999 (baseline) with those in 2007, a reduction in IPD rates of 37% and 92% was reported for all types and vaccine types, respectively [15].
Moreover, PCV7 has been demonstrated to be effective also for the prevention on non-invasive pneumococcal disease, particularly CAP. Data from the US Nationwide Inpatient survey system reported a 39% reduction in

hospital admissions for all-cause pneumonia in children aged < 2 years, and of 75% of pneumonia with *Sp* as aetiological agent: this resulted in fewer 41.000 admissions than the expected in the year 2004 [21]. These data are comparable with those reported in a recent survey, among children aged < 2 years, showing that the rate for all-cause pneumonia was approximately 35% lower in 2006 than during the control period 1997-1999, with the values of 9.1 per 1,000 and 8.1 per 1,000, in the period 2005-2006, respectively [22]. Another American study, comparing the pre-vaccination (1998-1999) and the post-vaccination period (2004), demonstrated a reduction of 52.4% and of 41.1% in the number of hospitalizations for all-cause pneumonia and of ambulatory visits for the same cause in young children, respectively [23].

Data obtained in Liguria, a North West Italian administrative Region, where PCV7 has been first introduced since 2003, are in accordance with the above mentioned results: during the period 2000-2007, a significant decline in hospitalization rates, among children aged 0-24 months, for all-cause and pneumococcal pneumonia ($p < 0.05$) was observed after the widespread immunization, with preventive fractions of 15% and 70.5%, respectively [24].

Recently, two studies, of particular interest, have investigated the capacity of pneumococcal conjugate vaccines to prevent hospitalisation for pneumonia in patients affected with seasonal influenza.

Madhi SA and colleagues, in a double-blind, randomized, placebo controlled trial in 39,836 children, 18,245 receiving all three doses of nine-valent pneumococcal conjugate vaccine (PCV9) and 18,268 receiving placebo, demonstrated that PCV9 was able to prevent 31% and 45% of hospitalisations for pneumonia associated with seven major respiratory viruses and influenza A virus, respectively [25].

An American research assessed the impact of infant immunization on pneumococcal pneumonia hospitalizations and mortality in all age groups using Health Care Utilization Project State Inpatient Databases (SID) from 1996 to 2006, in 10 US states. Compared to a 1996-1997 through 1998-1999 baseline, by the 2005-2006 season, both IPD and pneumococcal pneumonia hospitalizations and deaths had decreased substantially in all age groups, including a 47% reduction in non-bacteremic pneumococcal pneumonia in infants < 2 years old and a 54% reduction in adults > 65 years of age. Furthermore it has been estimated that, from 2000 to 2006, hospitalisations for pneumococcal pneumonia decreased of 788,838 cases: 90% of the reduction in pneumococcal pneumonia hospitalizations was attributed to the herd protection among adults 18 years old and older. Interestingly, in the first seasons after PCV7 introduction, when there were substantial state differences in coverage among subjects < 5-year-olds, US states with greater vaccination coverage had significantly fewer influenza-associated pneumonia hospitalizations among children, suggesting that this vaccine might also reduce influenza-attributable pneumonia hospitalisations, presumably because of its ability to prevent pneumococcal pneumonia frequently following influenza infection [26].

In Western Countries, deaths attributable to influenza has been significantly increased in the last 20 years, mainly due to the progressive aging of the population: in the frail elderly, the association of serious viral respiratory infections and bacterial co-infection, mainly attributable to *Sp*, represents a serious risk of death in these patients [4].

The positive results registered in children immunized with PCV7, in terms of protection against CAP and influenza-associated pneumonia, together with the possible availability of the new generation of pneumococcal conjugate vaccine (PCV13) in adults open interesting perspectives for improving the control of both *Sp*-associated diseases and influenza.

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