REVIEW

Improving the protection against *Streptococcus pneumoniae* with the new generation 13-valent pneumococcal conjugate vaccine

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Summary

The wide use of the 7-valent Pneumococcal Conjugate Vaccine (PCV7) determined, during the last decade, a dramatic decline in the incidence of Invasive Pneumococcal Diseases (IPD) in infants and children, and also among the non-vaccinated population through the phenomenon known as "herd protection". Furthermore a significant reduction of some non-IPD, such as Community Acquired Pneumonia (CAP) and Acute Otitis Media (AOM) was reported among the pediatric population. At the same time, the high vaccination coverage rates reached with PCV7 contributed to modify the ecology of Streptococcus pneumoniae (Sp), favoring the emergence of some serotypes not included in PCV7 and involved in IPD (replacement phenomenon), thus partially affecting the positive effects of the pediatric immunization programs. To remedy these shortcomings, a new generation of conjugate vaccines, with an enlarged antigenic spectrum of activity than PCV7, has been available since 2010. In particular, the 13-valent Pneumococcal Conjugate Vaccine (PCV13) has been authorized for active prevention of IPD, CAP and AOM in infants and children aged between 6 months and 5 years. More recently, in September 2011, the European Medicine Agency extended the indication for its use to include active immunization of adults aged ≥ 50 years for the prevention of IPD, thus opening new interesting opportunities to improve the control of pneumococcal disease among the entire population. The most interesting results from clinical trials using PCV13 in both children and adults are reported and discussed in details.

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Active immune prevention of *Streptococcus pneumoniae:* the milestones

The first attempts for developing a vaccine against *Streptococcus pneumoniae* (Sp) date from the beginning of 20th century, with the pioneering studies investigating the whole cell preparations. Subsequent investigations focused on live attenuated formulations and further studies led to the preparation of multivalent vaccines, containing partially purified capsular material, in the mid '30s. The first generation of polysaccharide vaccines became available in the late '40s.

The second generation of non-conjugated polysaccharide vaccines dates from the late '70s: a 14-valent vaccine was registered in the United States of America (US) in 1977, followed by a 23-valent formulation (PPV23) in 1983: even with some intrinsic immunological limitations, resulting in suboptimal efficacy to protect against Invasive Pneumococcal Diseases (IPD) and non-IPD, PPV23 has been targeted for adults belonging to some selected high risk categories and elderly, till today, and has been widely used worldwide, with million doses distributed.

To remedy these shortcomings, researchers have focused on the development of new types of vaccines against Sp: in particular, the formulations conjugating the superficial polysaccharide of the bacterium with specific carrier-proteins have undoubtedly marked a revolution in the active immune prevention of Sp-related diseases. The rational for this strategy lies in the ability of the carrier-protein to effectively stimulate the CD4+ T-cell dependent immune response, thus resulting in an optimal stimulation of the humoral immunity arm, the creation of a long term immunological memory against the presented antigens, and the secretion of specific mucosal IgA at the sites of entry of the bacterium into the organism [1, 2].

The first heptavalent Pneumococcal Conjugate Vaccine (PCV7) was licensed in the US in 2000, becoming available in Europe during the following year: it has been distributed in more than 70 Countries, with over 100 million of doses administered. This vaccine contains the polysaccharide antigens of the 7 serotypes (4, 6B, 9V, 14, 18C, 19F, 23F) responsible, in the pediatric setting, for more than 85% of the IPD in the US and for nearly 75% in Europe during the late '90s [2]. In the manufacturing of this vaccine, each polysaccharide antigen is conjugated with a carrier protein, named CRM197, a non-toxic mutant obtained from the purification of the toxin produced by the Corynebacterium diphtheriae.

As reported below in more details, PCV7 has been targeted for immunizing infants and children aged < 5 years, showing significant results against IPD in terms

of both efficacy and effectiveness. A further effect has been the significant reduction of the IPD even within the non-immunized individuals of all age-groups, an indirect phenomenon due to the relevant decline in the circulation of the microorganism in the community (herd effect). Moreover, an important reduction in the antibiotic resistance pattern has been documented following the wide clinical use of this vaccine in the pediatric age [2].
At the same time, numerous evidences have demonstrated the substantial impact of the PCV7 also against Community Acquired Pneumonia (CAP) and acute otitis media (AOM), two of the most relevant clinical pictures sustained by Sp [3-8].
Associated with the rapid and dramatic decline in the prevalence of the IPD caused by vaccine serotypes, the pneumococcal vaccination programs affected substantially the ecology of Sp. With respect to this, several reports showed an increase in the absolute frequency of IPD sustained by non-vaccine pneumococcal serotypes just in those Countries that adopted universal childhood immunization programs with PCV7. This phenomenon is known serotype replacement and has been involving numerous serotypes, such as 1, 3, 5, 6A, 7F, 15A, 22F, 33F, 35B, even if most of the evidences indicate that 19A serotype undoubtedly plays a major role worldwide [9-12].
As a matter of fact, a number of research projects has been carried out in order to develop new vaccines further improving the active immunoprophylaxis against the infections sustained by Sp. These efforts resulted in a new generation of conjugate vaccines, with enlarged antigenic spectrum of activity than the PCV7, available since 2010: a 10-valent vaccine (1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F), conjugated to protein D of H. influenzae and diphtheria and tetanus toxoids, (Synflorix®, GSK, Belgium), and a 13-valent CRM197-conjugated vaccine (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F), (Prevenar®, Wyeth, US). This latter has been initially targeted for the pediatric age, being its use possible also for the adults aged > 50 years since 2011.
For a more distant future, several experimental preparations are still under clinical development, and their prompt availability is not currently expected, at least in a short and medium term: mucosal-administered and, particularly, proteic vaccines, whose composition is based on specific Sp proteins, such as pneumolysin, choline binding proteins (PspA, PspC and Lyt A) and lipoproteins (PsaA), are at an early stage of development [13-15].

Direct and indirect effects of the universal children pneumococcal immunization programs with the PCV7

PCV7 was adopted in the US since 2000 for routine immunization of infants and young children and for older children at high-risk for developing IPD. The benefits of the introduction of the vaccine into the clinical use were substantial and almost immediate in the target pediatric population: according to data from the Active Bacterial Core Surveillance (ABCs), an active population- and laboratory-based surveillance system, the largest decline in the incidence rates by age class was recorded among children aged one year (-80%), a population showing the highest rates of morbidity prior to the vaccine licensure and its wide use [16].
Further data from the same national surveillance system, comparing the pre-vaccination (1998-1999) versus the post-vaccination era (2007), have confirmed the substantial decline in the incidence rate of IPD among the general population, with updated results showing a decline of 45% and 94% for cases sustained by all Sp-types and vaccine types, respectively [12, 17]. Particularly, monitoring a sample of the population of nearly 1.26 million subjects, a 100% and a 76% reduction in IPD rates, related to vaccine serotypes and by all Sp-types, were registered among children aged less than 5 years, comparing 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 [12, 17]. Interestingly, the universal vaccination of newborns immediately determined an indirect protection against IPD also among the non-vaccinated population, a phenomenon, known as "herd effect", already described for other infectious diseases: notably, in 2003, the vaccine prevented more than twice as many IPD cases in the US, just through this indirect effect [16]. 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 Sp-types and vaccine types, respectively [12]. This phenomenon was attributable to a reduced circulation of the Sp within the community, due to the reduction of the oropharyngeal carriage of the pathogen within the immunized population: this epidemiological scenario has been largely described in several studies published in the literature, being particularly marked in subjects aged > 50 years [16, 18-23].
The wide use of the PCV7 also determined important implications on the antibiotic resistance pattern of the most prevalent Sp serotypes circulating within the US community: the serotypes included in the vaccine formulation were just those most highly resistant to the different classes of antibiotics. A 81% reduction in the rate of IPD caused by penicillin-non-susceptible serotypes and a greater decline by 85% of IPD caused by macrolide-resistant serotypes were registered in US children aged < 2 years, following the wide introduction of pneumococcal vaccination [24-26]. As a further consequence of the herd effect, a parallel decrease of antibiotic resistance occurred even in older children and among elderly [24].
Similar results concerning the effects of the PCV7 against IPD have been also described outside the US, thus further confirming the positive effects of the universal children immunization campaign.
In Canada, where PCV7 has been recommended for routine vaccination since 2002, a broad active surveillance,

conducted among children aged 16 years and younger, by 12 centers of the Canadian Immunization Monitoring Program demonstrated that, during the period 2000-2007, the absolute number of reported IPD cases decreased 48% ($p < 0.01$) over the 8-year period and 56% ($p < 0.01$) in children 0-4 years of age, with a 87.5% ($p < 0.01$) and 92% ($p < 0.01$) reduction of IPD cases sustained by vaccinal serotypes, reported overall and in children 0-4 years, respectively [27].

Favorable findings have been registered also in the European Countries which adopted a universal strategy with PCV7, according to different immunization schedules, either with three (i.e., a 2+1 simplified doses regimen) or four doses given to children during the first two years of life: for example, an effectiveness of 72% and 74% against IPD was reported among the target population in England and Wales [28] and in Norway [29], respectively.

Similarly, a significant reduction in the incidence of IPD has also been recently demonstrated in France, where PCV7 has been introduced in the national immunization schedule since 2003: cases of meningitis and sepsis in children aged < 2 years decreased from 9.3/100,000 to 5.4/100,000 and from 22.4/100,000 to 16.6/100,000, respectively [30]. In the same Country, a 82% reduction in the incidence of pneumococcal meningitis in 0-24 month children, from 8.9 cases per 100,000 population in 2001 to 1.8 cases per 100,000 population in 2005 ($p = 0.03$), was documented by others [31].

The first report of the effectiveness of PCV7 in Denmark, where a 2+1 schedule was adopted demonstrated that, 1 year following its introduction in the national childhood immunization program, the overall incidence of IPD, overall and by vaccine-serotypes, rapidly declined from 54 to 23 cases per 100,000 and from 36.7 to 7.7 cases per 100,000 in children aged < 2 years, respectively [32].

In Italy, PCV7 became available in October 2001: the Ministry of Health initially recommended the free of charge offer of the vaccine only for children affected by specific risk conditions (sickle cell anemia, thalassemia, asplenia, etc.), aged < 5 years [33]. Liguria was the first Italian administrative Region, since 2003, to actively recommend the free of charge immunization of all infants with the PCV-7, within a research pilot-project. Vaccination coverages among infants rapidly increased from 42.8% in 2003 to 83.3% in 2004, progressively reaching levels of 93.4% in 2007. A scientific project, aimed at evaluating the effect of the vaccination campaign, showed, during the period 2000-2007, a significant decline in hospitalization rates, among children aged 0-24 months, for all-cause and pneumococcal pneumonia ($p < 0.05$) and for acute otitis media ($p < 0.01$), with preventive fractions of 15%, 70.5% and 36.4%, respectively [8]. These results clearly showed the effectiveness of PCV-7 in the prevention of these mucosal non-invasive pneumococcal diseases and are broadly in line with other on-field studies performed on larger study population.

Of particular interest, with respect to 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 etiological agent: this resulted in fewer 41,000 admissions than the expected in the year 2004 [3]. These data are superimposable 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 [4]. 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, respectively [5].

Similarly, after the PCV7 introduction, also the US national rates for AOM outpatient visits significantly declined by 20% in children aged < 2 years [6]. Other authors described a 42.7% decrease in the number of outpatient visits for the same clinical outcome in the same target population [7]. Notably, the reduction in the incidence of AOM resulted in the consequent decline of related surgery interventions, like pressure-equalizing tube insertions, confirming the relevant morbidity of this microorganism [34]. This is consistent with the fact that pneumococcal AOM may present with more severe clinical signs and symptoms than AOM caused by either H. influenzae and M. catharralis [35]. Moreover, these data are well within the range of vaccine-efficacy (10-50%) demonstrated by the long-term follow up of the Finnish Otitis Media (FinOM) and Northern California Kaiser Permanente (NCKP) clinical trials against recurrent episodes or for the prevention of tympanostomy tube placement [36].

Rational for the introduction of 13-valent pneumococcal vaccine: the serotype replacement

Before introduction of PCV7, pneumococcal serotypes contained in the vaccine were responsible for nearly 80% of IPD in the US, 70% of the IPD in Europe, and 40-80% in other Regions of the world [2]. Although the dramatic impact, in terms of reduction in both the incidence of IPD and non-IPD, determined by the wide use of PCV7, the potential for vaccination to contribute to the emergence of serotypes not included in the vaccine, the so-called "replacement" phenomenon, thus reducing the positive effect of vaccination, has been a concern for researchers. Following the introduction of PCV7, several studies have identified some non-PCV7 serotypes causing IPDs (i.e., 1, 3, 5, 6A, 7F, 15A, 19A, 22F, 33F, 35B) as emerging serotypes; some of them have shown a significant increase, with 19A being the most prevalent. Data from ABCs indicated that the overall incidence of IPD among children aged < 5 years decreased from approximately 99 cases per 100,000 subjects during 1998-1999 to 21 cases per 100,000 in 2008 (percentage reduction: 79%). The reduction in overall IPD resulted

from a 99% decrease in disease caused by the seven serotypes contained in PCV7 and serotype 6A, a serotype against which PCV7 provided cross-protection, but these decreases have been partially offset by increases in IPD caused by non-vaccine serotypes, in particular 19A [37].

Interestingly, a 15-year prospective surveillance study of all culture-proven invasive infections determined by Sp in infants and children, hospitalized in 8 children's hospitals in the US, demonstrated that, since the implementation of routine PCV7 immunization in 2000, IPD have decreased yearly from 2001 through 2004. The rate of IPD then increased from 2005 through 2008, primarily as a result of serotype 19A isolates. Notably, in 2007 and 2008, only 16 isolates (4%) were vaccine serotypes, while 19A accounted for 46% of the non-PCV7 serotypes and serotypes 1, 3, and 7F accounted for further 22% of the non-PCV7 serotypes. Overall, compared with the total number of annual admissions, the number of 19A isolates increased significantly from 2001 to 2008 ($P < 0.00001$) [38]. Further results, obtained from 30,032 cases laboratory-confirmed IPD cases, including 5410 among children aged < 5 years, identified in US during 1998-2007 period by 8 active population-based surveillance sites adhering to ABCs, confirmed this trend among general population. In 2006-2007, PCV7 serotypes accounted for only 2% of all IPD among children aged < 5 years, compared with 83% at baseline. The proportions of IPD cases caused by PCV7-type strains decreased from 56% to 10% and from 56% to 9% among 18-64-year-old and ≥ 65-year-old adults, respectively. Overall and PCV7-type IPD incidence declined by 45% (from 24.4 to 13.5 cases per 100,000 population) and 94% (from 15.5 to 1.0 cases per 100,000 population), respectively ($P < 0.01$). By contrast, the incidence of IPD caused by serotype 19A and other non-PCV7 types increased from 0.8 to 2.7 cases per 100,000 individuals and from 6.1 to 7.9 cases per 100,000 individuals, respectively ($P < 0.01$) [12].

Similar findings have been obtained for pneumococcal meningitis. An active population-based surveillance from eight sites in the US, investigating the changes in the incidence of pneumococcal meningitis from 1998 through 2005, demonstrated that the rates of pneumococcal meningitis have decreased among children and adults since PCV7 was introduced, but the increase in meningitis caused by non-PCV7 serotypes partially reduced this result. In more details, overall incidence of pneumococcal meningitis declined from 1.13 cases to 0.79 case per 100,000 persons between 1998-1999 and 2004-2005 (a 30.1% decline, $P < 0.001$), and rates of PCV7-serotype meningitis declined from 0.66 case to 0.18 case per 100,000 individuals (a 73.3% decline, $P < 0.001$) among patients of all ages. However the reduction of meningitis incidence caused by PCV7-related-serotype was less marked (32.1%, $P = 0.08$), while the rates of non-PCV7-serotype disease even increased from 0.32 to 0.51 per 100,000 persons (an increase of 60.5%, $P < 0.001$), with the most important growth involving serotypes 19A, 22F, and 35B [11].

The emergence of non-PCV7 serotypes, in particular 19A, has been recently described also in Europe. The results of typization of 282 Sp, isolated in 2007 from adults and children affected by IPD in France, showed that serotype 19A was the most frequently isolated serotype both in children and adults. In particular, 19A represented 13.0% and 28.6% of the strains isolated from IPD in adults and children, respectively. In this latter population, the most frequent serotypes isolated in IPD, following 19A, were 1 and 3 in 19.0% and 9.0% of the cases, respectively. In adults, Sp serotypes involved in IPD were more heterogeneous than in children: the four most prevalent serotypes representing, together with 19A, 50% of the isolated strains were 9 (11.0%), 3 (9.6%), 7F (8.2%), and 14 (8.2%) [39].

Also in Norway, 19A has been identified as a major cause of IPD following the introduction of PCV7. From 2004-2005 to 2008, even if the incidence rate of IPD in general population decreased from 24.05 cases/100,000 population in 2004-2005 to 18.09 cases/100,000 population in 2008, IPD caused by serotype 19A increased from 0.26 to 0.70 cases/100,000 population. Differently from the US, where serotype 19A has also emerged as the most frequent cause of drug-resistant IPD, the clinical forms caused by penicillin non-susceptible pneumococci (PNSP) serotype 19A has remained unchanged in Norway; moreover the proportion of PNSP serotype 19A among all 19A serotypes decreased from 2004-2005 to 2008, but this change was non statistically significant [40].

With respect to bacteremic pneumonia, an observational study, performed in Italy from April 2007 through June 2009, among 292 children aged 0-16 years admitted with a diagnosis of CAP to 83 pediatric hospitals in Italy, identified Sp in 80 of 292 patients. Serotype 1 resulted the most frequent serotype (32.5%) and was significantly associated with complications and older age, serotype 19A was second in frequency (15.0%) and was significantly associated with younger age; serotype 3 was isolated in 12.5% of the cases [41].

In Liguria, after PCV7 implementation since 2003, the global proportion of PCV7 serotype out of all Sp responsible for IPD declined from 38% in 2006-08 to 10% in 2009-10, compared to the proportion of 51% reported by a nationwide surveillance performed before the introduction of the conjugate vaccine. Among non-PCV7 serotypes, the proportion of type 1, 7F and 19A increased in the period 2006-2010, although no type reached a proportion higher than 15%. In the post-vaccination period, the role of type 3, 6C and 19A has been important also among serotypes that cause non-IPD, with proportions ranging between 7 and 15% [42].

The introduction of PCV7 vaccination in Liguria has also affected the nasopharyngeal carriage of Sp. A cross-sectional study, performed collecting nasopharyngeal swab from 699 children aged < 5 years, reported that among PCV7 serotypes, serotype 4 was carried by 8.2% (age-weighted carriage prevalence, 95% Confidence Interval – 95%IC = 4.3-12.1%) of the population and accounted for 72.2% of all PCV7 isolates. Among non-PCV7 serotypes, 5 and 19A showed a higher preva-

lence, being carried by 15.2% (95%CI = 10.1-20.3%) and 8.8% (95%CI = 4.8-12.8%) of the population, respectively [43].

Overview of the 13-valent pneumococcal conjugate vaccine phase 3 clinical development and introduction of the 13-valent pneumococcal conjugate vaccine in the national childhood vaccination schedules

To address the replacement phenomenon, a 13-valent pneumococcal conjugate vaccine (PCV13) has been developed in order to improve the protection against pneumococcal disease. Actually, according to the known serotype prevalence, 90% or more of the invasive pneumococcal disease in most regions of the world should be preventable with the use of PCV13 vaccine. However, on the basis of guidelines by the World Health Organization (WHO) and the requirements of national regulatory authorities, the licensing of new pneumococcal vaccines required randomized clinical trials to demonstrate the non-inferiority of PCV13 compared to existing pneumococcal conjugate vaccines.

Details about the clinical development plan of PCV 13 are outlined in Tab. I. In particular, since in many countries PCV7 was administered concomitantly with other infant vaccinations, including diphtheria-tetanus-acellular pertussis-hepatitis B-inactivated polio-Haemophilus influenzae type B (DTaP-HBV-IPV/Hib) vaccine, it was necessary to demonstrate that the administration of PCV13, concomitantly with the other routine infant vaccines, was safe, well tolerated and did not interfere with the immune response against the concomitant vaccine antigens.

A phase III clinical trial was performed in Italy to compare the safety and immunogenicity of PCV13 with those of PCV7 when administered concomitantly with DTaP-HBV-IPV/Hib vaccine. 606 infants were enrolled and randomly assigned to receive either PCV13 or PCV7 at 3, 5, and 11 months of age subjects, as recommended by the national pediatric vaccination schedule. The immunogenicity of the vaccines, measured 1 month after the two-dose primary series and 1 month after the toddler dose, was evaluated in terms of antibody responses to DTaP-HBV-IPV/Hib antigens, and serotype-specific anticapsular polysaccharide IgG responses and antipneumococcal opsonophagocytic assay (OPA), in a subset group of infants, for pneumococcal vaccines. The response to DTaP-HBV-IPV/Hib antigens was comparable with both PCV13 and PCV7. PCV13 elicited antipneumococcal capsular IgG antibodies to all 13 vaccine serotypes, with marked increases in concentrations registered after the toddler dose. Although a lower immunogenicity for serotypes 6B and 23F was observed after the primary series of PCV13, responses to the seven common serotypes were comparable between the PCV13 and PCV7 groups when measured after the toddler dose. Moreover, PCV13 was able to elicit substantial levels of OPA activity against all 13 serotypes following both the infant series and the toddler dose. In conclusion, the results of this phase III clinical trial indicated that PCV13 could be administered as part of a routine infant immunization program implemented by a 2 + 1 schedule [44].

Similar findings have been obtained when PCV13 was administered according to a 4-dose series in infants, and as a toddler dose in infants previously vaccinated with PCV7. In a phase III clinical trial, a total of 613 healthy subjects aged 2 months were enrolled and randomly assigned to receive either PCV13 or PCV7 at 2, 3, and 4 months concomitantly with pentavalent vaccine containing diphtheria, tetanus, 2-pertussis components, inactivated poliovirus, and Hib vaccine antigens. At 12 months of age (toddler dose), all subjects received the toddler dose of pentavalent vaccine and children assigned in the PCV13 group received a toddler dose of PCV13 (PCV13/PCV13 group), whilst half of the subjects in the PCV7 group received a toddler dose of PCV7 (PCV7/PCV7 group), and the other half received one dose of PCV13 (PCV7/PCV13 group). Anti-pneumococcal im-

Tab. I. Overview of the 13-valent pneumococcal conjugate vaccine (PCV13) phase 3 clinical development.

Non Inferiority Studies	
Evaluation of different dosing schedules	
«3 + 1» Schedule	2, 3, 4 & 12-15 months 2, 4, 6 & 12-15 months
«2 + 1» Schedule	2, 4 & 12 months 3, 5 & 11 months
«3 + 0» Schedule	6, 10 & 14 weeks
Older children (never vaccinated): 1, 2 or 3 doses	
Transition study (PCV13 following 7-valent pneumococcal conjugate vaccine)	
Immune response to concomitantly administered vaccine	Hexavalent-, pentavalent-, DTwP-, MMR-, MMRV-, MenC-, HAV-, HBV-Vaccines and OPV
Safety evalutation in all studies	

mune responses were studied by measuring serotype-specific anticapsular polysaccharide IgG antibodies 1 month after the infant series in PCV13 recipients and before the toddler dose and 1 month after the toddler dose in all subjects. OPA against the 6 additional serotypes (1, 3, 5, 6A, 7F, and 19A) were performed, after the toddler dose, in a subset of 100 subjects from the PCV13/PCV13 and PCV7/PCV13 study groups. Following the infant dose series, PCV13 resulted able to elicit similar immune response for the 7 serotypes in common to both PCV13 and PCV7. For these 7 common serotypes, the immunogenicity were comparable across all the study groups also after the toddler dose. With respect to the six additional serotypes, the immune response elicited by the infant doses of PCV13 was similar to that measured against the common serotypes. A robust response to the 6 additional serotypes were also elicited by the toddler dose of PCV13 toddler dose both after PCV13 and PCV7 infant series. The capability of a single toddler dose of PCV13 to potentially provide protection against the 6 additional serotypes is further supported by the observed elicitation of functional OPA responses [45].

On the basis of the above mentioned results, PCV13 vaccination has been implemented since 2010 in several countries, according to different vaccinations schedules providing recommendations also for the administration of PCV13 in children that had already received one or more doses of PCV7. In Italy, PCV13 has been initially authorized for the active prevention of IPD, pneumonia and acute otitis media in infants and children aged between 6 months and 5 years. According to the Italian vaccination schedule (Fig. 1), non-vaccinated infants should be received 3 doses of PCV13 at 3, 5 and 12 months concomitantly with the three doses of DTaP-HBV-IPV/Hib vaccine. Non-vaccinated children aged between 12 and 24 months and above to 24 months, should receive two doses of PCV13 at intervals of two months and a single dose of vaccine, respectively. Infants and children who started immunization with PCV7 may complete it by switching to PCV13 at any stage of the vaccination schedule [46]. Similar schedules have been adopted also in other European countries such as France and UK.

In US, the primary infant series consists of 3 doses of PCV13, administered at 2,4 and 6 months at intervals of approximately 8 weeks, and the booster dose is recommended at age 12-15 months and at least 8 weeks after the third dose. Healthy children aged 7-59 months who have not been vaccinated with PCV7 or PCV13 previously should receive 1 to 3 doses of PCV13, depending on their age at the time when vaccination begins and whether underlying medical conditions are present. Infants and children aged < 24 months who have received ≥ 1 dose of PCV7 should complete the vaccination series with PCV13. Children aged 12-23 months who have received 3 doses of PCV7 before age 12 months are recommended to receive 1 dose of PCV13, administered at least 8 weeks after the most recent dose of PCV7. Finally, 1 dose of PCV13 is recommended for all healthy children aged 24-59 months with any incomplete PCV schedule (PCV7 or PCV13) before age 24 months [37].

Adult vaccination with the pneumococcal conjugate vaccines: looking to the future with the new generation 13-valent conjugate formulation

Sp is responsible for significant morbidity and mortality worldwide in adults, related both to IPD and non-IPD. In particular, pneumococcal CAP determine a significant impact in older adults because of its relatively high incidence rate, the associated economic cost, and their high case-fatality rates. However a precise estimation of the burden of pneumococcal pneumonia is challenging since the diagnosis of pneumonia can be confused with several other common infectious and non-infectious diseases, including bronchitis, congestive heart failure, pulmonary infarction, and atelectasis. Furthermore, the etiological agent of pneumonia is not identified for the great majority of cases, and thus the proportion of all pneumonia cases caused by Sp remains uncertain.

Recently, some interesting studies have assessed the burden of IPD and pneumococcal pneumonia among older adults both in US and Europe. Weycker et al. estimated that among the 91.5 million US adults aged ≥ 50 years, 29,500 cases IPD (27,700 cases of bacteremia

Fig. 1. 13-valent pneumococcal conjugate vaccine Italian vaccination schedule for healthy infants (a) and infants affecting by risk conditions for invasive pneumococcal disease (b).

a

3 Months	5 Months	11 Months	16-24 Months
PCV13	PCV13	PCV13	---
PCV7	PCV13	PCV13	---
PCV7	PCV7	PCV13	---
PCV7	PCV7	PCV7	PCV13

b

3 Months	5 Months	11 Months	12-15 Months	16-24 Months
PCV13	PCV13	PCV13	---	---
PCV7	PCV13	PCV13	PCV13	---
PCV7	PCV7	PCV13	PCV13	---
PCV7	PCV7	PCV7	PCV13	PCV13

PCV13: 13-valent pneumococcal conjugate vaccine
PCV7: 7-valent pneumococcal conjugate vaccine

and 1,800 cases of meningitis), 502,600 cases of non-bacteremic pneumococcal pneumonia (198,600 cases requiring hospital care and 304,000 cases treated outside the hospital setting), and 25,400 pneumococcal-related deaths (6,200 due to invasive disease and 19,200 due to pneumonia) are estimated to occur yearly. In terms of direct and indirect costs, this important clinical impact has been translated into an annual estimation of 3.7 and 1.8 billion dollars respectively, most of which (81% direct and 62% indirect) are attributable to non-bacteriemic pneumococcal pneumonia [47].

The impact of CAPs among older adults has been investigated also within the European context, where the total annual rate ranged between 1.6 and 12/1000, including both hospitalized and non-hospitalized cases. A recent study, performed in Germany on a large national database including the entire adult population, based on the analysis of the hospital discharge chart including diagnoses for CAP (n = 388,406), showed an annual incidence of this clinical picture equal to 2.75 and 2.96/1000 inhabitants in 2005 and 2006, respectively. The mean incidence of CAP, during the study period, resulted 7.65/1000 in patients aged > 60 years [48].

Moreover, a recent review, analyzing the clinical and economic burden, etiology and resistance patterns of CAP in European adults, demonstrated that the incidence of CAP varied by country, age and gender, and was higher in individuals aged ≥ 65 years and in men; related mortality varied from < 1% to 48% and was associated with advanced age, co-morbidity conditions and CAP severity. Sp resulted the most common agent isolated [49]. Furthermore, Sp plays an important role in determining secondary bacterial pneumonia in case of both influenza epidemics and pandemics, representing an important cause of excess mortality. The synergism between influenza virus and Sp was first suggested by studies performed on samples collected during autopsy from victims of 1918 influenza pandemic, and has been recently confirmed by data collected during the 2009 A/H1N1v influenza pandemic [50].

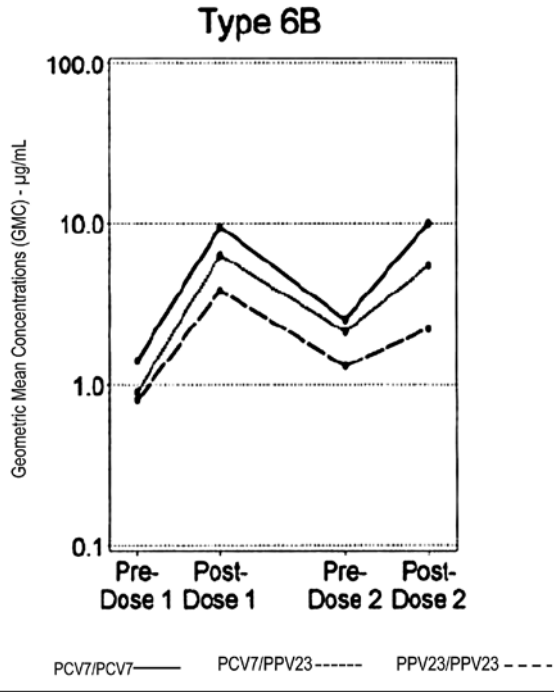
The severe burden of Sp among adults together with the controversial efficacy of the PPV23, particularly in those subject at highest risk for IPD and pneumonia, and its immunological inability to adequately stimulate the T-cell dependent immune response, resulting in the failure to create an immunological memory, has recently led to investigate the immunogenicity of pneumococcal conjugate vaccines in adults and elderly, when administered either as primary or booster dose.

A pivotal dose-ranging study, performed among 220 elderly aged 70-79 who had received the PPV23 at least 5 years prior to enrollment and assigned to receive one of four volumes (0.1, 0.5, 1 or 2 ml) of PCV7 or a 0.5 ml dose of PPV23, showed that the administration of a 1 ml dose of PCV7 vaccine is more immunogenic than 0.5 ml of PPV23 vaccine in elderly adults previously vaccinated with PPV23; the vaccines were comparable safe and well tolerated by the study-subjects [51].

Another study compared the immunogenicity of PCV7, in terms of antipolysaccharide enzyme-linked immunosorbent assay antibody concentrations and OPA, with that of PPV23 within 220 adults aged > 70 years who had not been previously vaccinated with a pneumococcal vaccine. One year after the vaccination, the subjects vaccinated with PCV7 received a booster dose of either PCV7 or PPV23, while all subjects immunized with PPV23 received a booster dose of PCV7. The results demonstrated that an initial dose of PCV7 was likely to elicit higher and potentially more effective levels of antipneumococcal antibodies than those measured after primary immunization with PPV23. Interestingly, the initial dose of PCV7 created an immunological response that permits subsequent administration of PCV7 or PPV23 to maintain functional antipolysaccharide antibody levels, while the initial dose of PPV23 induced a condition of hyporesponsiveness, thus the successive administration of PCV7 induced significantly lower antibacterial responses for all serotypes, compared with those induced by PCV7 alone [52]. The antipolysaccharide enzyme-linked immunosorbent assay antibody concentrations against type 6B for the 3 cohorts of subjects, evaluated after both the first and second doses of vaccine, are summarized in Figure 2.

More recently, a randomized, double-blind clinical study compared the immunogenicity, in terms of OPA titer, of a single dose of PCV13 with PPV23 in 835 adults aged 60-64 years and the immunogenicity of PCV13 in 404 adults aged 50-59 years compared to that measured within the 60-64 years study group. Among adults 60-64 years, 1 month following vaccination, OPA titers in the PCV13 group resulted significantly higher than in the PPV23 group in 8 out of 12 serotypes common to both

Fig. 2. Antipneumococcal polysaccharide binding antibody responses against Streptococcus pneumoniae type 6B, elicited during the immunization series (from De Roux et al., 2008 [52], mod.).



vaccines (1, 4, 6B, 7F, 9V, 18C, 19A and 23F) and for 6A (not contained in the PPV23), while it was comparable for the other 4 common serotypes (3, 5, 14 and 19F). With respect to the 50-59 years study group, 1 month following vaccination, OPA titers were significantly higher than in subjects aged 60-64 years for nine serotypes and were comparable for the other four serotypes. In both age groups OPA titers declined from one month to one year after PCV13 administration, but remained higher than baseline titers. These results clearly support a potential benefit of PCV13 in pneumococcal vaccine naïve older adults [53].

A randomized, double-blind study, performed among 938 adults > 70 years of age, previously vaccinated with a single dose of PPV23 at least five years prior to enrollment, evaluated the immunogenicity of PCV13 in comparison with PPV23. One year after the enrollment vaccination (PCV13 or PPV23), all subjects received a dose of PCV13. Blood samples were obtained prior to and at one month after each vaccination and tested for OPA titers. Among enrolled subjects, at 1 month after the enrollment vaccination, OPA titers in the PCV13 group were significantly higher than in the PPV23 group in 10 out of 12 serotypes common to both vaccines (1, 4, 5, 6B, 7F, 9V, 18C, 19A, 19F and 23F) and for 6A (not contained in the PPV23), whilst it was comparable for the other 2 common serotypes (3 and 14). Furthermore, OPA titers increased in response to the PCV13 given at one year, but the responses to the dose of PCV13 given at one year were generally higher in the group that received PCV13 at enrollment compared with the group that received PPV23 at enrollment [54].

Since pneumococcal vaccine and seasonal influenza vaccine are commonly recommended for older adults, the possibility to administer concomitantly both vaccines is an important topic to investigate in order to facilitate immunization. Compatibility of the PPV23 co-administered with the influenza vaccine has been demonstrated previously. Recently, a randomized, double-blind clinical study has evaluated the safety and immunogenicity of PCV13 when administered concomitantly with the Trivalent inactivated Influenza Vaccine (TIV) in adults aged ≥ 65 years who are naïve to PPV23. The 1160 enrolled subjects were randomized 1:1 to receive PCV13+TIV followed by placebo, or Placebo+TIV followed by PCV13 at 0 and 1 months. Blood samples were drawn at 0, 1, and 2 months and tested for serotype-specific anticapsular polysaccharide immunoglobulin G geometric mean concentrations. Slightly lower anticorpal titers were observed with PCV13+TIV relative to PCV13, but overall concomitant PCV13+TIV demonstrated acceptable immunogenicity and safety compared with either agent given alone, thus opening the possibility to co-administer the two vaccines [55].

On the basis of these results obtained in the clinical development plan of PCV13 when administered in adults aged 50 years, the European Medicine Agency, in September 2011, extended the indication to use of PCV 13 to include active immunization of adults aged ≥ 50 years against IPD [56].

The positive results, in terms of immunogenicity, obtained with pneumococcal conjugate vaccines in adults need to be confirmed as effectiveness in the prevention of IPD and CAP. In this view, a clinical trial, entitled "Community Acquired Pneumonia Immunization Trial in Adults", has been performing in order to establish the efficacy of PCV13 vaccine in the prevention of a first episode of vaccine-serotype specific pneumococcal CAP among 85,000 Dutch community-dwelling adult persons aged ≥ 65 years [57]. Preliminary results of the study should be available soon and the definitive results will be critical to determine the position of conjugate pneumococcal vaccines in the prevention of pneumococcal disease.

In conclusion, the availability of a new generation of conjugate pneumococcal vaccines with an enlarged antigenic spectrum (i.e. PCV13), together with the excellent results obtained worldwide with PCV7, in terms of effectiveness against the different clinical forms attributable to Sp, offer promising perspectives to improve prevention and control of IPD and non-IPD, particularly CAP, not only in children, but also among adults.

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