Streptococcus pneumoniae oropharyngeal colonization in school-age children and adolescents with type 1 diabetes mellitus: Impact of the heptavalent pneumococcal conjugate vaccine

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This study evaluated *Streptococcus pneumoniae* colonization in children and adolescents with type 1 diabetes mellitus (DM1) to investigate the theoretical risk of invasive pneumococcal disease (IPD) in these patients and the potential protective efficacy of pneumococcal conjugate vaccines (PCVs). An oropharyngeal swab was obtained from 299 patients aged 6–17 y with DM1 who were enrolled during routine clinical visits. DNA from swabs was analyzed for *S. pneumoniae* using real-time polymerase chain reaction. *S. pneumoniae* was identified in the swabs of 148 subjects (49.8%). Colonization was strictly age-related and declined significantly in the group aged ≥ 15 years (odds ratio [OR] 0.28; 95% confidence interval [CI], 0.14–0.57). Carriage was also significantly influenced by sex (lower in females: OR 0.56; 95% CI, 0.35–0.91), ethnicity (less common among non-Caucasians: OR 0.34; 95% CI, 0.13–0.89), parental smoking habit (more frequent among children with at least one smoker between parents: OR 1.76; 95% CI, 0.90–2.07), and the administration of antibiotic therapy in the previous 3 months (less frequent among patients who received antibiotics: OR 0.21; 95% CI, 0.07–0.62). Multivariate analyses of the entire study population showed no association between carriage and PCV7 vaccination status. Serotypes 19F, 9V, and 4 were the most frequently identified serotypes. In conclusion, school-age children and adolescents with DM1 are frequently colonized by *S. pneumoniae*, and protection against pneumococcal carriage following infant and toddler vaccination was not effective after several years. Together with the need to increase vaccine uptake in all the children aged < 2 years, these results suggest that PCV booster doses are needed in DM1 patients to maintain the protection offered by these vaccinations.

Introduction

Infections in patients with type 1 diabetes mellitus (DM1) are significantly more common and severe than in healthy subjects, and infections cause several medical, social and economic problems.^{1–3} Infections due to *Streptococcus pneumoniae* remain a relevant problem despite improvements in specific preventive and therapeutic measures. One study in England³ demonstrated that the introduction of the 7-valent pneumococcal conjugate vaccine (PCV7) into the pediatric immunization schedule reduced invasive pneumococcal disease (IPD)

incidence in vaccinated and unvaccinated subjects,⁴ but the hospitalization rates of patients with DM1 for IPD remained more than 3 times higher than healthy subjects. This result explains the long-standing policies in many countries to offer pneumococcal vaccines to diabetics.^{5,6} The United States also strongly recommends vaccination in children aged 6–18 y with DM1.⁷ The immunization schedule particularly suggests the administration of the 23-valent pneumococcal polysaccharide vaccine (PPSV23) in patients who previously received PCV7 and/or the 13-valent conjugate vaccine (PCV13) without a further booster dose of PPSV23.⁷

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Pneumococcal pharyngeal carriage is a prerequisite for the development of IPD,⁸ and the reduction of colonization by invasive serotypes is associated with a reduction in the incidence of IPD in vaccinated and unvaccinated subjects.⁹ PPSV23 does not influence pneumococcal colonization,¹⁰ but all of the conjugate vaccines (PCVs) reduce the carrier state of the serotypes included in each vaccine.¹¹ Consequently, PCVs are more effective than PPSV23, and PCVs may be used alone or in combination with PPSV23 for IPD prevention in at-risk children aged 6–18 years, including children with DM1. However, no data on the real immunogenicity and clinical efficacy of PCVs in children with DM1 are available. Pneumococcal colonization in these patients could be evaluated to overcome this limitation and collect information on the potential efficacy of PCVs.¹² Moreover, the monitoring of pneumococcal colonization in school-aged children and adolescents, who are regularly vaccinated with a PCV in the first year of life and who did not receive any further pneumococcal vaccination, may indicate the duration of protection against colonization and offer suggestions for the adequate timing of booster doses.

This study evaluated *S. pneumoniae* colonization in a group of school-aged children and adolescents with DM1 to determine the theoretical risk of IPD in these patients and the potential protective efficacy of the PCV against the largest number of serotypes (i.e., PCV13). Moreover, the first pneumococcal conjugate vaccine, PCV7, was not widely used in Italy until 2009, even in children with a chronic severe underlying disease, and it was administered to less than 50% of the infant population.¹³ Therefore, carriage evaluation of children born before 2009 may enable carriage comparisons in vaccinated and unvaccinated DM1 pediatric patients and measurement of the long-term impact of PCV7.

Results

Table 1 shows the characteristics of the 299 enrolled children with DM1 (153 males, 51.29%; mean age $\pm$ standard deviation, 12.6 ± 3.1 years). According to the common age distribution of DM1 in pediatric age, 62 (20.7%) children were aged <10 years, 155 (51.8%) children were aged 10–14 years, and 82 (27.4%) children were aged ≥ 15 years. *S. pneumoniae* was identified in the swabs of 148 subjects (49.8%). All the children had a well-controlled DM1. The prevalence of carriers was significantly higher in children aged <10 y and aged 10–14 y than children aged ≥ 15 years (25.7% and 56.1% vs. 18.2%; $p < 0.001$). Carriers were significantly more often male than female (58.8% vs. 41.2%; $p = 0.009$), and carriers more commonly had at least one smoker parent compared with non-carriers (36.5% vs. 25.2%; $p = 0.03$). There were no differences between carriers and non-carriers in ethnicity, number of siblings, gestational age, birth weight, exclusive breast feeding, allergic sensitization, meningococcal or influenza vaccination.

Table 2 shows the associations between demographic and clinical characteristics and pneumococcal carriage. Colonization was strictly age-related, and it declined significantly in the group

aged ≥ 15 years (OR 0.28; 95% CI, 0.14–0.57). Colonization was significantly influenced by sex (lower in females: OR 0.56; 95% CI, 0.35–0.91), ethnicity (less common among non-Caucasians: OR 0.34; 95% CI, 0.13–0.89), parental smoking habit (more frequent in children with at least one smoking parent: OR 1.76; 95% CI, 0.90–2.07), and the administration of antibiotic therapy in the previous 3 months (less frequent in patients who received antibiotics: OR 0.21; 95% CI, 0.07–0.62). By contrast, the presence of siblings (OR 0.97; 95% CI, 0.55–1.72), the use of drugs other than antibiotics (OR 0.94; 95% CI, 0.44–2.00), and the presence of concurrent endocrinological disease (OR 0.91; 95% CI, 0.38–2.19) did not influence carrier state.

Table 3 shows the relationship between pneumococcal vaccination status and pneumococcal carriage in the population as a whole and the 2 younger age groups (there were too few vaccinated children in the group aged ≥ 15 years). A total of 110 children were fully vaccinated with PCV7 according with the official recommendations. The last dose of PCV7 was administered, on average, about 8 y before the study period in children <10 years, about 11.5 y in those aged 10–14 years, and about 12 y in those ≥ 15 years. None of the enrolled subjects have received PPSV23. The proportions of carriers in the entire population of any pneumococcal serotype, any serotype included in PCV7 and the 6 additional serotypes included in PCV13 were similar in PCV7 vaccinated and unvaccinated subjects. Multivariate analyses revealed no association between carriage and PCV7 vaccination status: OR 0.74 (95% CI, 0.44–1.27) for carriers of any pneumococcal serotype; 0.74 (95% CI, 0.44–1.26) for carriers of any serotype in PCV7; and 0.52 (95% CI, 0.26–1.04) for carriers of any of the 6 additional serotypes included in the PCV13. Sub-analysis of the group aged <10 y revealed similar results, with no differences between vaccinated and unvaccinated subjects. By contrast, data of children aged 10–14 y showed that the additional PCV13 serotypes were significantly more frequent in PCV7 unvaccinated than vaccinated children (28.1% vs. 7.0%; OR 0.20; 95% CI, 0.06–0.65).

Table 4 shows the individual serotypes identified by vaccination status. A total of 18.2%, 18.2%, and 12.7% of the vaccinated children colonized by *S. pneumoniae* were carriers of one, 2 or ≥ 3 PCV13 serotypes, respectively. The corresponding figures in unvaccinated patients were 14.2%, 22.2% and 13.1%, respectively, with no significant difference between groups. Serotypes 19F, 9V, and 4 were the most frequently identified serotypes in vaccinated and unvaccinated patients. None of the serotypes included in PCV7 or PCV13 was associated with PCV7 vaccination status.

Discussion

This study demonstrated that the prevalence of pneumococcal colonization in children and adolescents with DM1 was age-related, higher in males, more frequent in patients exposed to passive smoking and lower in patients who recently received antibiotic therapy. These results were expected because male gender and passive smoking were previously reported risk factors for

Table 1. Main characteristics of 299 children and adolescents with type 1 diabetes mellitus by pneumococcal carriage

	All children (n = 299)	Carriers (n = 148)	Non-carriers (n = 151)	p-value
Age (years)				
<10	62 (20.7)	38 (25.7)	24 (15.9)	<0.001
10–14	155 (51.8)	83 (56.1)	72 (47.7)	
≥15	82 (27.4)	27 (18.2)	55 (36.4)	
Sex				
Male	153 (51.2)	87 (58.8)	66 (43.7)	0.009
Female	146 (48.8)	61 (41.2)	85 (56.3)	
Ethnicity				
Caucasian	276 (92.3)	141 (95.3)	135 (89.4)	0.06
Non-Caucasian	23 (7.7)	7 (4.7)	16 (10.6)	
No. of siblings ^a				
0	67 (22.6)	35 (23.6)	32 (21.5)	0.78
1	149 (50.2)	72 (48.7)	77 (51.7)	
2	63 (21.2)	33 (22.3)	30 (20.1)	
≥3	18 (6.1)	8 (5.4)	10 (6.7)	
Parental smoking habit				
Both non-smokers	207 (69.2)	94 (63.5)	113 (74.8)	0.03
At least one smoker	92 (30.8)	54 (36.5)	38 (25.2)	
Gestational age (weeks)				
<37	33 (11.0)	17 (11.5)	16 (10.6)	0.81
≥37	266 (89.0)	131 (88.5)	135 (89.4)	
Birth weight (g) ^a				
<2,500	14 (4.9)	9 (6.3)	5 (3.4)	0.25
≥2,500	274 (95.1)	133 (93.7)	141 (96.6)	
Exclusive breastfeeding ^a				
No	87 (29.2)	42 (28.6)	45 (29.8)	0.82
Yes	211 (70.8)	105 (71.4)	106 (70.2)	
Allergy				
No	227 (75.9)	115 (77.7)	112 (74.2)	0.48
Yes	72 (24.1)	33 (22.3)	39 (25.8)	
Allergic sensitization				
No	233 (77.9)	117 (79.0)	116 (76.8)	0.64
Yes	66 (22.1)	31 (21.0)	35 (23.2)	
Meningococcal vaccination ^a				
No	127 (44.1)	61 (42.4)	66 (45.8)	0.55
Yes	161 (55.9)	83 (57.6)	78 (54.2)	
Influenza vaccination during current season				
No	194 (64.9)	96 (64.9)	98 (64.9)	0.99
Yes	105 (35.1)	52 (35.1)	53 (35.1)	

^aSome missing values.

pneumococcal colonization in randomly selected children and adolescents aged 10–19 y¹⁴. A progressive reduction in pneumococcal colonization with increasing age is a common finding in otherwise healthy children.^{15,16} This observation is ascribed to the continuous exposure to circulating pneumococcal serotypes that evokes protective immunity and the reduced role of risk factors, such as day-care attendance, which can significantly enhance the horizontal spread of pneumococcal strains. The reduction of colonization in patients recently treated with antibiotics is explained by the evidence that most of the drugs used to treat common pediatric infections maintain sufficient activity against *S. pneumoniae* to modify carriage significantly, despite the recent increase in the prevalence of pneumococcal resistance to antibiotics.¹⁷

By contrast, the very high rate of pneumococcal carriage found in the population of school-aged children and adolescents with DM1 was completely unexpected. In this study, the total number of children with Afro-Caribbean ethnicity, a group of

subjects at increased risk of pneumococcal infections, was marginal. Consequently, ethnicity cannot be considered a factor able to explain this finding.¹⁸ Similar conclusions can be drawn for the presence of siblings, a condition that is usually associated with greater colonization rates¹⁸ but that in this study was not associated with any modification of pneumococcal colonization of the enrolled children. The high colonization rate, higher than the rates found in otherwise healthy subjects of the same age in the studies performed with culture or PCR of nasopharyngeal swabs,¹⁸ may be explained by the fact that we used the most accurate available methods to identify pneumococcal carriage. Respiratory secretions were collected from the oropharynx (which is the most effective site for detecting *S. pneumoniae* in older children and adults)¹⁹ and we used a flocced nylon-fiber tip (because previous studies demonstrated that this tip ensures the highest rate of detection of *S. pneumoniae*, particularly compared with the more widely used Dacron and rayon swabs).²⁰ *S. pneumoniae* was identified using molecular methods that are more sensitive

Table 2. Association between demographic and clinical characteristics and pneumococcal carriage in children and adolescents with type 1 diabetes mellitus

	Carriers (n=148)	Non-carriers (n=151)	OR (95% CI) Univariate	OR (95% CI) ^a Multivariate
Age (years)				
<10	38 (25.7)	24 (15.9)	1 (reference)	1 (reference)
10–14	83 (56.1)	72 (47.7)	0.73 (0.40–1.33)	0.66 (0.35–1.23)
≥15	27 (18.2)	55 (36.4)	0.31 (0.16–0.62)	0.28 (0.14–0.57)
Sex				
Male	87 (58.8)	66 (43.7)	1 (reference)	1 (reference)
Female	61 (41.2)	85 (56.3)	0.54 (0.34–0.86)	0.56 (0.35–0.91)
Ethnicity				
Caucasian	141 (95.3)	135 (89.4)	1 (reference)	1 (reference)
Non-Caucasian	7 (4.7)	16 (10.6)	0.42 (0.17–1.05)	0.34 (0.13–0.89)
Siblings ^b				
No	35 (23.6)	32 (21.5)	1 (reference)	1 (reference)
Yes	113 (76.3)	117 (78.5)	0.88 (0.51–1.52)	0.97 (0.55–1.72)
Parental smoking habit				
Both non-smokers	94 (63.5)	113 (74.8)	1 (reference)	1 (reference)
At least one smoker	54 (36.5)	38 (25.2)	1.71 (1.04–2.81)	1.76 (1.04–2.97)
Antibiotic therapy (last 3 months) ^b				
No	141 (96.6)	131 (87.3)	1 (reference)	1 (reference)
Yes	5 (3.4)	19 (12.7)	0.24 (0.09–0.67)	0.21 (0.07–0.62)
Other therapies (last 3 months) ^b				
No	131 (89.1)	133 (88.7)	1 (reference)	1 (reference)
Yes	16 (10.9)	17 (11.3)	0.96 (0.46–1.97)	0.94 (0.44–2.00)
Concurrent endocrinological disease ^b				
No	136 (92.5)	136 (91.3)	1 (reference)	1 (reference)
Yes	11 (7.5)	13 (8.7)	0.85 (0.37–1.96)	0.91 (0.38–2.19)

CI: confidence interval; OR: odds ratio. ^aMultivariate models included terms for age, gender, ethnicity, siblings, and parental smoking plus, in turn, each clinical characteristic analyzed; ^bThe sums do not add up to the total because of a few missing values.

than traditional non-enriched cultures that are used in routine practice, albeit with some exceptions.^{21,22}

However, immune factors may have influenced colonization rates. Several studies demonstrated that host defenses are impaired in patients with DM1, and this deficiency may produce several clinical problems, including a higher pneumococcal colonization rate. The reduction of immunity in patients with DM1 involves innate and adaptive immune system functions.^{23–25} All

phagocyte functions, adherence, chemotaxis, phagocytosis, and bactericidal activity are significantly impaired, and dysfunction of dendritic cells, reduced T-cell response, and depressed bactericidal serum activity were also demonstrated.^{26–28} These immune defects favor increased pharyngeal colonization rates because intact innate and T-cell-mediated immunity, especially the Th17 subset of T helper cells, are essential for the effective clearance of nasopharyngeal colonization.^{29–31} Moreover, systemic

Table 3. Relationship between pneumococcal vaccination status and pneumococcal carriage in children with type 1 diabetes mellitus^{ab}

	Vaccinated with PCV7 (n = 110)	Not vaccinated against pneumococcus (n = 176)	OR (95% CI)
Pneumococcal carrier status			
Carriers, any serotype	56 (50.9)	88 (50.0)	0.74 (0.44–1.27)
Carriers, serotypes in PCV7	54 (49.1)	86 (48.9)	0.74 (0.44–1.26)
Carriers, 6 additional serotypes in PCV13	15 (13.6)	37 (21.0)	0.52 (0.26–1.04)
Sub-group aged <10 years	(n = 37)	(n = 23)	
Carriers, any serotype	21 (56.8)	16 (69.6)	0.29 (0.07–1.23)
Carriers, serotypes in PCV7	21 (56.8)	15 (65.2)	0.42 (0.11–1.56)
Carriers, 6 additional serotypes in PCV13	7 (18.9)	6 (26.1)	0.51 (0.11–2.30)
Sub-group aged 10–14 years	(n = 57)	(n = 89)	
Carriers, any serotype	27 (47.4)	54 (60.7)	0.62 (0.30–1.29)
Carriers, serotypes in PCV7	25 (43.9)	54 (60.7)	0.53 (0.26–1.10)
Carriers, 6 additional serotypes in PCV13	4 (7.0)	25 (28.1)	0.20 (0.06–0.65)

CI: confidence interval; OR: odds ratio; PCV7: 7-valent pneumococcal conjugate vaccine; 13-valent pneumococcal conjugate vaccine. ^aORs adjusted for age (using a continuous term), gender, ethnicity, number of siblings, and parental smoking. The reference categories for the analyses are non carriers of, respectively, 1) any serotype, 2) serotypes in PCV7 and 3) 6 additional serotypes in PCV13; ^bno information concerning the pneumococcal vaccination status of 13 children.

Table 4. Carriage of specific pneumococcal sub-types in children with type 1 diabetes mellitus by pneumococcal vaccination status^a

	Vaccinated with PCV7 (n = 110)	Not vaccinated against pneumococcus (n = 176)	OR (95% CI) ^b
Carrier status			
Positive for serotype 1	2 (1.8)	7 (4.0)	0.34 (0.06–1.79)
Positive for serotype 3	1 (0.9)	3 (1.7)	0.40 (0.04–4.29)
Positive for serotype 4	15 (13.6)	23 (13.1)	0.81 (0.38–1.70)
Positive for serotype 5	9 (8.2)	16 (9.1)	0.90 (0.36–2.21)
Positive for serotype 6A	1 (0.9)	7 (4.0)	0.18 (0.02–1.60)
Positive for serotype 6B	0 (0.0)	0 (0.0)	NE
Positive for serotype 7F	0 (0.0)	1 (0.6)	NE
Positive for serotype 9V	18 (16.4)	28 (15.9)	0.85 (0.42–1.70)
Positive for serotype 14	0 (0.0)	1 (0.6)	NE
Positive for serotype 18C	0 (0.0)	0 (0.0)	NE
Positive for serotype 19A	6 (5.4)	7 (4.0)	1.68 (0.50–5.59)
Positive for serotype 19F	53 (48.2)	83 (47.2)	0.82 (0.49–1.39)
Positive for serotype 23F	0 (0.0)	0 (0.0)	NE

CI: confidence interval; NE: not estimable; OR: odds ratio; PCV7: 7-valent pneumococcal conjugate vaccine. ^aThirteen subjects had missing information on pneumococcal vaccination. ^bORs adjusted for age (using a continuous term), gender, ethnicity, number of siblings, and parental smoking. Reference category is non-carrier of each corresponding serotype.

and mucosal anti-pneumococcal antibodies in healthy children prevent the new acquisition of *S. pneumoniae* in the nasopharynx.^{32,33} The high colonization rate found in patients with DM1 may explain the higher risk of IPD documented in these subjects because pneumococcal carriage is a pre-requisite for IPD development,^{1–3} and this result suggests that all preventive measures to contain pneumococcal carriage should be used to reduce this risk. PPSV23 does not influence carriage, and it cannot reduce IPD risk through this mechanism. Therefore, PCV administration may be the best solution to reduce IPD risk. Unfortunately, PCVs are based on T-dependent antigens that seem to evoke a lower immune response lower in patients with DM1 than healthy children. Eibl et al. tested a hepatitis A vaccine and diphtheria toxoid in patients with DM1 and found that the primary antibody response to T-cell-dependent antigens and the T-cell response to primary protein antigens was reduced in these subjects, and only an additional booster immunization could overcome the defect.²⁶ No data on the immunogenicity of PCVs in DM1 patients are presently available, and it is not known whether the impaired immune response of these subjects is sufficient to persistently reduce colonization and the timing of booster dose administration. Further studies are needed to investigate these factors. However, the need for further specifically planned studies to evaluate the potential protective effects of PCVs and its duration against carriage are suggested by our data on the types of colonization in the patients with DM1 in this study. No significant differences in the carriage of pneumococcal serotypes included in PCV7 were found between children ≥ 6 years who previously received or did not receive this vaccine. Only one sample was taken from each child, and we did not evaluate pneumococcal colonization over time. However, our results suggest that the efficacy of PCV7 against colonization does not persist until school age in children who are immunized in the first years of life and later developed DM1, at least when vaccine administration is relatively low and greater than 50% of

classmates remain unvaccinated. A similar finding was recently reported in a study of otherwise healthy school-aged children and adolescents,¹⁶ and the lack of PCV efficacy was considered a consequence of the progressive decrease over time of the antibody concentrations that were evoked in the first year of life but were no longer adequate to avoid colonization at the beginning of the school age. Each serotype has different putative correlates of protection,³⁴ and it is likely that the serotypes that need the highest concentrations to be eliminated are more frequently carried. This hypothesis was confirmed in our study in otherwise healthy subjects¹⁶ because the most commonly detected serotype was 19F, which has a very high correlate of protection against IPD.³⁵ Conversely, Simell et al. studied the association between pneumococcal nasopharyngeal carriage and serum concentrations of serotype-specific antibodies in toddlers one month after immunization with a 9-valent PCV³⁴ and found that higher anti-serotypes 14 and 19F IgG and anti-serotype 14 IgM correlated with a lower probability of pneumococcal acquisition. The re-colonization with pneumococcal serotypes that were included in the previously used PCV7 indicates that a booster dose should be administered before entering school in order to maintain the prevention of colonization with these serotypes. However, the timing and number of booster doses have not been defined, and these factors must be established by further specifically planned studies that have to take in account also the problem of pediatric vaccination coverage and the indirect effect of pediatric PCV administration. As previously mentioned, this study was carried out in an area where pediatric pneumococcal vaccination coverage was only about 50%. Because this limited coverage could have only marginally reduced circulation of pneumococcal serotypes included in PCV7 and its indirect effect, it is possible that increasing vaccination coverage a more prolonged impact on pharyngeal colonization could take place. These factors seem particularly important in DM1 subjects for whom the above mentioned reduction of immune system functions likely requires a specific immunization schedule.^{23–31}

More than 95% of the children colonized by *S. pneumoniae* carried at least one of the serotypes included in PCV13. The number, types and clinical relevance of non-PCV13 serotypes colonizing children carrying PCV13 strains are not known, but our results suggest that PCV13 plays a relevant role in the prevention of IPD in children with DM1.

In conclusion, this study demonstrated that school-age children and adolescents with DM1 were frequently colonized by *S. pneumoniae*, and protection against pneumococcal carriage from infant and toddler vaccination was not effective after several years. Together with the need to increase vaccine uptake in all the children aged <2 years, these results suggest that PCV booster doses in DM1 patients are needed to maintain the protection offered by these vaccines. However, the number of booster doses to maintain long-term protection and the timing of administration are not known. Moreover, the need for association of PCV with the PPSV23 in high-risk patients including those with DM1 has to be determined. Specific studies at these factors are needed because of the immune system dysfunctions in patients with DM1.

Methods

Patient enrolment and swab collection

The Ethics Committees of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, and the other participating hospitals (sited in Modena, Rome, Verona, Chieti, and Novara) approved the protocol of this multicentre study. Children and adolescents were enrolled after written parental consent, and written assent was also obtained from subjects aged ≥8 years.

Patients aged 6–17 y with DM1 diagnosed according to the criteria established by the American Diabetes Association³⁶ and who regularly attended the pediatric diabetes center of each hospital between January 2014 and December 2014 entered the study. Exclusion criteria were hemoglobin A_{1c} (Hb A_{1c}) >8.5%, severe diabetic microvascular or macrovascular complications, primary or secondary immune deficiency, any active infection, administration of antibiotics in the previous 2 weeks, and pathological routine blood tests other than glucose or Hb A_{1c}. The clinical and laboratory data of each enrolled child for the previous 3 months were retrieved from their clinical records and recorded in an electronic database specifically prepared for the study.

Patients' pneumococcal vaccination status was established by consultation with the official vaccination chart issued by the Vaccination Service of the Region in which the patients lived. In Italy vaccines are given free of charge and administered by Regional Vaccination Services in more than 95% of the cases and immediately recorded in electronic charts. Consequently, consultation with these charts permits to collect complete information on vaccination status of any subject. The pneumococcal immunization schedule that was recommended by the Italian Ministry of Health before 2011, which is the period that enrolled subjects could have received pneumococcal vaccine, involved 3 doses of PCV7 in the first year of life, or 2 in the second year, or a single

dose after the second year until the fifth year.³⁷ Patients were considered fully vaccinated with PCV7 if one of these recommendations was fulfilled by the time of enrolment, and patients were not fully vaccinated if they had started, but not completed, the vaccine schedule. The latter group consisted of only 1% of the enrolled subjects, and this group was not compared with the groups of fully vaccinated or unvaccinated children. Information regarding the use of the PPSV23 was also collected addressing specific questions to the parents and to the patients themselves when older than 10 y of age.

Oropharyngeal samples were obtained using an ESwab kit that contained a polypropylene screw-capped tube filled with 1 mL of liquid Amies medium (Brescia, Copan, Italy). Sampling was performed by pressing the tongue downward to the floor of the mouth with a spatula, and tonsillar arches and the posterior nasopharynx were swabbed without touching the sides of the mouth. All swabs were immediately refrigerated at –20°C and transported within one week to the central laboratory, where they were processed within 2 hours of arrival.

Identification of *S. pneumoniae*

Bacterial genomic DNA was extracted from the samples (250 µL) using a NucliSENS easyMAG automated extraction system (BioMérieux, Bagnoli a Ripoli, Florence, Italy) and a generic protocol. Samples were tested for the autolysin-A-encoding (*lytA*) and the *wzg* (*cpsA*) genes of *S. pneumoniae* using real-time polymerase chain reaction (PCR) as previously described.³⁸ The detection level of this test was 16 genome copies, and each sample was tested in triplicate. Samples were considered positive if at least 2 of the 3 tests revealed the presence of both genes. No internal amplification control was used in the reaction to maximize sensitivity, but an external control was performed. Real-time PCR-negative specimens were also tested for the presence of an RNase P-encoding gene to exclude PCR inhibition and DNA extraction failure.

All positive cases were serotyped using primers and probes that were designed using GenBank database sequences (www.ncbi.nlm.nih.gov) of serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F (i.e., serotypes in the 13-valent pneumococcal conjugate vaccine, PCV13), and synthesized by TIB Molbiol (Genoa, Italy) as previously described.³⁹ Analytical specificity was pre-evaluated using computer-aided analyses in Primer-blast (www.ncbi.nlm.nih.gov/tools/primer-blast) and BLAST software (www.blast.ncbi.nlm.nih.gov/Blast.cgi) to compare the collected sequences with all of the sequences listed under 'bacteria' and 'Homo sapiens'.

Statistical analysis

Groups were compared using the χ^2 or Fisher's exact test as appropriate. Ordered categorical data were compared using a Cochran-Armitage test for trends. Multivariate odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using unconditional multiple logistic regression models to measure associations between i) pneumococcal vaccination and pneumococcal carrier status and ii) selected demographic and clinical characteristics and pneumococcal carrier status. Adjustments were made

for the following covariates, which were defined *a priori*, on the basis of the available literature and potential confounding issues: age, gender, ethnicity, number of siblings, and parental smoking habits. Stratified analyses were also used for age subgroups (<10, 10–14, and >15 years), to address the potential role of age as an effect modifier of the association. All analyses were 2-tailed, and *p*-values of 0.05 or less were considered statistically significant. All analyses were performed using SAS version 9.2 (Cary, NC, USA).

Disclosure of Potential Conflicts Of Interest

No potential conflicts of interest were disclosed.

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References

- Muller LM, Gorter KJ, Hak E, Goudzwaard WL, Schellevis FG, Hoepelman AI, Rutten GE. Increased risk of common infections in patients with type 1 and type 2 diabetes mellitus. *Clin Infect Dis* 2005; 41:281-8; PMID:16007521; <http://dx.doi.org/10.1086/431587>
- Kornum JB, Thomsen RW, Riis A, Lervang HH, Schønheyder HC, Sørensen HT. Diabetes, glycemic control, and risk of hospitalization with pneumonia: a population-based case-control study. *Diabetes Care* 2008; 31:1541-5; PMID:18487479; <http://dx.doi.org/10.2337/dc08-0138>
- Seminog OO, Goldacre MJ. Risk of pneumonia and pneumococcal disease in people hospitalized with diabetes mellitus: English record-linkage studies. *Diabet Med* 2013; 30:1412-9; PMID:23796064; <http://dx.doi.org/10.1111/dme.12260>
- Gladstone RA, Jefferies JM, Faust SN, Clarke SC. Continued control of pneumococcal disease in the UK - the impact of vaccination. *J Med Microbiol* 2011; 60:1-8; PMID:20965923; <http://dx.doi.org/10.1099/jmm.0.020016-0>
- Department of Health, Joint Committee on Vaccination and Immunisation. Immunisation Against Infectious Disease. London: Her Majesty's Stationery Office (HMSO), 1992. Department of Health, Joint Committee on Vaccination and Immunisation. Immunisation against infectious disease. London: Her Majesty's Stationery Office (HMSO), 1992.
- Centers for Disease Control and Prevention. Prevention of Pneumococcal Disease. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 1997; 46 (No. RR-8):1-25; PMID:9011775
- Kim DK, Bridges CB, Harriman KH, Centers for Disease Control and Prevention (CDC), Advisory Committee on Immunization Practices (ACIP), ACIP Adult Immunization Work Group. Advisory Committee on Immunization Practices recommended immunization schedule for adults aged 19 years or older—United States, 2015. *MMWR Morb Mortal Wkly Rep* 2015; 64:91-2; PMID:25654609
- Simell B, Auranen K, Käyhty H, Goldblatt D, Dagan R, O'Brien KL. The fundamental link between pneumococcal carriage and disease. *Expert Rev Vaccines* 2012; 11:841-55; PMID:22913260; <http://dx.doi.org/10.1586/erv.12.53>
- Davis SM, Deloria-Knoll M, Kassa HT, O'Brien KL. Impact of pneumococcal conjugate vaccines on nasopharyngeal carriage and invasive disease among unvaccinated people: review of evidence on indirect effects. *Vaccine* 2013; 32:133-45; PMID:23684824; <http://dx.doi.org/10.1016/j.vaccine.2013.05.005>
- Veenhoven R, Bogaert D, Uiterwaal C, Brouwer C, Kiezebrink H, Bruin J, Jzerman E, Hermans P, de Groot R, Zegers B, Kuis W, Rijkers G, Schilder A, Sanders E. Effect of conjugate pneumococcal vaccine followed by polysaccharide pneumococcal vaccine on recurrent acute otitis media: a randomised study. *Lancet* 2003; 361:2189-95; PMID:12842372; [http://dx.doi.org/10.1016/S0140-6736\(03\)13772-5](http://dx.doi.org/10.1016/S0140-6736(03)13772-5)
- Rinta-Kokko H, Dagan R, Givon-Lavi N, Auranen K. Estimation of vaccine efficacy against acquisition of pneumococcal carriage. *Vaccine* 2009; 27:3831-7; PMID:19490983; <http://dx.doi.org/10.1016/j.vaccine.2009.04.009>
- Auranen K, Rinta-Kokko H, Goldblatt D, Nohynek H, O'Brien KL, Satzke C, Simell B, Tanskanen A, Käyhty H; Pneumococcal Carriage Group (PneumoCarr). Colonisation endpoints in *Streptococcus pneumoniae* vaccine trials. *Vaccine* 2013; 32:153-8; PMID:24016803; <http://dx.doi.org/10.1016/j.vaccine.2013.08.061>
- Istituto Superiore di Sanità. Centro Nazionale di Epidemiologia, Sorveglianza e Promozione della Salute. Dati e evidenze disponibili per l'utilizzo dei vaccini anti-pneumococchi nei soggetti a rischio di qualsiasi età e per l'eventuale ampliamento dell'offerta ai soggetti anziani. Available at: <http://www.epicentro.iss.it/temi/vaccinazioni/pdf/Dati%20e%20evidenze%20vaccini%20antipneumococchi.pdf>. Accessed on March 19, 2015.
- Cardozo DM, Nascimento-Carvalho CM, Andrade AL, Silvany-Neto AM, Daltro CH, Brandão MA, Brandão AP, Brandileone MC. Prevalence and risk factors for nasopharyngeal carriage of *Streptococcus pneumoniae* among adolescents. *J Med Microbiol* 2008; 57:185-9; PMID:18201984; <http://dx.doi.org/10.1099/jmm.0.47470-0>
- Mehr S, Wood N. *Streptococcus pneumoniae* - a review of carriage, infection, serotype replacement and vaccination. *Paediatr Respir Rev* 2012; 13:258-64; PMID:23069126; <http://dx.doi.org/10.1016/j.prrv.2011.12.001>
- Principi N, Terranova L, Zampiero A, Montinaro V, Ierardi V, Peves Rios E, Pelucchi C, Esposito S. Pharyngeal colonisation by *Streptococcus pneumoniae* in older children and adolescents in a geographical area characterised by relatively limited pneumococcal vaccination coverage. *Pediatr Infect Dis J* 2015; 34:426-32; PMID:25760567; <http://dx.doi.org/10.1097/INF.0000000000000637>
- Esposito S, Principi N. Pharmacotherapy for pneumococcal infections: an update. *Expert Opin Pharmacother* 2013; 14:65-77; PMID:23256539; <http://dx.doi.org/10.1517/14656566.2013.756867>
- Le Polain de Waroux O, Flasche S, Prieto-Merino D, Prieto-Merino D, Edmunds WJ. Age-dependent prevalence of nasopharyngeal carriage of *Streptococcus pneumoniae* before conjugate vaccine introduction: a prediction model based on a meta-analysis. *PLoS One* 2014; 9:e86136; PMID:24465920; <http://dx.doi.org/10.1371/journal.pone.0086136>
- Principi N, Terranova L, Zampiero A, Manzoni F, Senatore L, Rios WP, Esposito S. Oropharyngeal and nasopharyngeal sampling for the detection of adolescent *Streptococcus pneumoniae* carriers. *J Med Microbiol* 2014; 63:393-8; PMID:24298049; <http://dx.doi.org/10.1099/jmm.0.068726-0>
- Dube FS, Kaba M, Whittaker E, Zar HJ, Nicol MP. Detection of *Streptococcus pneumoniae* from different types of nasopharyngeal swabs in children. *PLoS One* 2013; 8: e68097; PMID:23840817; <http://dx.doi.org/10.1371/journal.pone.0068097>
- Carvalho MG, Pimenta FC, Moura I, Roundtree A, Gertz RE Jr, Li Z, Jagero G, Bigogo G, Junghae M, Conklin L, et al. Non-pneumococcal mitis-group streptococci confound detection of pneumococcal capsular serotype-specific loci in upper respiratory tract. *Peer J* 2013; 1:e97.99; PMID:23825797; <http://dx.doi.org/10.7717/peerj.97>
- Cvitkovic Spik V, Beovic B, Pokorn M, Drole Torkar A, Vidmar D, Papst L, Seme K, Kogoj R, Müller Premru M. Improvement of pneumococcal pneumonia diagnostics by the use of RT-PCR on plasma and respiratory samples. *Scand J Infect Dis* 2013; 45:731-7; PMID:23826792; <http://dx.doi.org/10.3109/00365548.2013.804631>

23. Balasoju D, van Kessel KC, van Kats-Renaud HJ, Collet TJ, Hoepelman AI. Granulocyte function in women with diabetes and asymptomatic bacteriuria. *Diabetes Care* 1997; 20:392-5; PMID:9051393; <http://dx.doi.org/10.2337/diacare.20.3.392>
24. Delamaire M, Maugeudre D, Moreno M, Le Goff MC, Allanic H, Genetet B. Impaired leucocyte functions in diabetic patients. *Diabetes Med* 1997; 14:29-34; [http://dx.doi.org/10.1002/\(SICI\)1096-9136\(199701\)14:1%3c29::AID-DIA300%3e3.0.CO;2-V](http://dx.doi.org/10.1002/(SICI)1096-9136(199701)14:1%3c29::AID-DIA300%3e3.0.CO;2-V)
25. Jakelic J, Kokic S, Hozo I, Maras J, Fabijanic D. Non-specific immunity in diabetes: Hyperglycemia decreases phagocytic activity of leukocytes in diabetic patients. *Med Arch* 1995; 49:9-12
26. Eibl N, Spatz M, Fischer GF, Mayr WR, Samstag A, Wolf HM. Impaired primary immune response in type-1 diabetes: results from a controlled vaccination study. *Clin Immunol* 2002; 103:249-59; PMID:12173299; <http://dx.doi.org/10.1006/clim.2002.5220>
27. Spatz M, Eibl N, Hink S, Wolf HM, Fischer GF, Mayr WR, Scherthaner G, Eibl MM. Impaired primary immune response in type-1 diabetes. Functional impairment at the level of APCs and T-cells. *Cell Immunol* 2003; 221:15-26; PMID:12742378; [http://dx.doi.org/10.1016/S0008-8749\(03\)00043-1](http://dx.doi.org/10.1016/S0008-8749(03)00043-1)
28. Peleg AY, Weeraratna T, McCarthy JS, Davis TM. Common infections in diabetes: pathogenesis, management and relationship to glycaemic control. *Diabetes Metab Res Rev* 2007; 23:3-13; PMID:16960917; <http://dx.doi.org/10.1002/dmrr.682>
29. van Rossum AM, Lysenko ES, Weiser JN. Host and bacterial factors contributing to the clearance of colonization by *Streptococcus pneumoniae* in a murine model. *Infect Immun* 2005; 73:7718-26; PMID:16239576; <http://dx.doi.org/10.1128/IAI.73.11.7718-7726.2005>
30. Malley R, Trzcinski K, Srivastava A, Thompson CM, Anderson PW, Lipsitch M. CD4+ T cells mediate antibody-independent acquired immunity to pneumococcal colonization. *Proc Natl Acad Sci USA* 2005; 102:4848-53; PMID:15781870; <http://dx.doi.org/10.1073/pnas.0501254102>
31. Lu YJ, Gross J, Bogaert D, Finn A, Bagrade L, Zhang Q, Kolls JK, Srivastava A, Lundgren A, Forte S, et al. Interleukin-17A mediates acquired immunity to pneumococcal colonization. *PLoS Pathog* 2008; 4:e1000159; PMID:18802458; <http://dx.doi.org/10.1371/journal.ppat.1000159>
32. Zhang Q, Bernatoniene J, Bagrade L, Pollard AJ, Mitchell TJ, Paton JC, Finn A. Serum and mucosal antibody responses to pneumococcal protein antigens in children: relationships with carriage status. *Eur J Immunol* 2006; 36:46-57; PMID:16342325; <http://dx.doi.org/10.1002/eji.200535101>
33. Dagan R, Givon-Lavi N, Fraser D, Lipsitch M, Siber GR, Kohberger R. Serum serotype-specific pneumococcal anticapsular immunoglobulin g concentrations after immunization with a 9-valent conjugate pneumococcal vaccine correlate with nasopharyngeal acquisition of pneumococcus. *J Infect Dis* 2005; 192:367-76; PMID:15995949; <http://dx.doi.org/10.1086/431679>
34. Simell B, Nurkka A, Lahdenkari M, Givon-Lavi N, Käyhty H, Dagan R, Jokinen J. Association of serotype-specific antibody concentrations and functional antibody titers with subsequent pneumococcal carriage in toddlers immunized with a 9-valent pneumococcal conjugate vaccine. *Clin Vaccine Immunol* 2012; 19:96-9; PMID:22072724; <http://dx.doi.org/10.1128/CVI.05369-11>
35. Andrews NJ, Waight PA, Burbidge P, Pearce E, Roalfe L, Zancolli M, Slack M, Ladhani SN, Miller E, Goldblatt D. Serotype-specific effectiveness and correlates of protection for the 13-valent pneumococcal conjugate vaccine: a postlicensure indirect cohort study. *Lancet Infect Dis* 2014; 14:839-46; PMID:25042756; [http://dx.doi.org/10.1016/S1473-3099\(14\)70822-9](http://dx.doi.org/10.1016/S1473-3099(14)70822-9)
36. American Diabetes Association. Standard of medical care in diabetes – 2013. *Diabetes Care* 2013; 36 Suppl. 1:S11-S66; PMID:23264422
37. Ministero della Salute. Piano Nazionale Vaccini 2005–2007. Available at: http://www.salute.gov.it/portale/documentazione/p6_2_2_1.jsp?lingua=italiano&cid=543. Accessed on March 16, 2015.
38. Marchese A, Esposito S, Coppo E, Rossi GA, Tozzi A, Romano M, Da Dalt L, Schito GC, Principi N. Detection of *Streptococcus pneumoniae* and identification of pneumococcal serotypes by real-time polymerase chain reaction using blood samples from Italian children ≤5 years of age with community-acquired pneumonia. *Microb Drug Resist* 2011; 17:419-24; PMID:21510746; <http://dx.doi.org/10.1089/mdr.2011.0031>
39. Esposito S, Marchese A, Tozzi AE, Rossi GA, Da Dalt L, Bona G, Pelucchi C, Schito GC, Principi N; Italian Pneumococcal CAP Group. Bacteremic pneumococcal community-acquired pneumonia in children less than 5 years of age in Italy. *Pediatr Infect Dis J* 2012; 31:705-10; PMID:22426300; <http://dx.doi.org/10.1097/INF.0b013e31825384ae>