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Studying PCV impact on clinical presentation of otitis media helps to understand its pathogenesis

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ABSTRACT

Background: Complex otitis media (OM) may present with intact tympanic membrane or spontaneous otorrhea. We compared dynamics of intact tympanic membrane and spontaneous otorrhea OM following 7- and 13-valent conjugated vaccines (PCV7, PCV13) implementation, since differences in dynamics may imply different underlying mechanisms.

Methods: A prospective, population-based, active surveillance. Episodes with middle-ear fluid cultures in children < 3 years were included. Defined sub-periods were: pre-pneumococcal conjugated vaccines (PCV) (2004–2008); PCV7 (2009–2011); PCV13 (2014–2016).

Results: Of 7705 episodes, 57.2% had intact tympanic membrane, 16.8% spontaneous otorrhea, 26.0% unknown.

In the pre-PCV period, the spontaneous otorrhea group was older and had higher proportions of factors associated with recurrence/chronicity.

During the PCV7 period, spontaneous otorrhea and intact tympanic membrane episodes caused by PCV13 serotypes decreased significantly (43% and 51%, respectively) and those caused by non-PCV13 serotypes and culture-negative episodes increased significantly. However, rates increases were steeper in the spontaneous otorrhea group for both non-PCV13 serotypes (117% *vs.* 38%) and culture-negative (720% *vs.* 69%). In the spontaneous otorrhea group, nontypeable *Haemophilus influenzae* rates increased non-significantly by 10% and all-cause OM rates increased significantly by 56%, while in the intact tympanic membrane group the respective rates decreased significantly by 22% and 11%. These trends were especially pronounced in ages 24–35 months.

Despite these differences, after PCV13 introduction, both spontaneous otorrhea and intact tympanic membrane rates declined for all outcomes.

Conclusions: Spontaneous otorrhea was associated with older age, frequent history of complex OM and delayed PCV impact, suggesting a higher proportion of advanced-stage complex OM.

1. Introduction

Otitis media (OM) is a multifactorial, often polymicrobial disease [1–3]. Clinical manifestations range from early, uncomplicated, sometimes asymptomatic OM (simple OM) to complex OM such as recurrent, non-responsive, spontaneously draining and OM with effusion [1].

OM disease spectrum is wide, and it is not always feasible to distinguish between early acute OM (which probably presents in early infancy in most cases) and complex OM. Nevertheless, complex OM is associated with the heaviest disease burden, and with several features, including (but not limited to) frequent, recurrent, nonresponsive to antibiotic treatment, and chronic episodes. Consequently, complex OM episodes are also associated with middle ear fluid (MEF) cultures obtainment, through tympanocentesis or from spontaneously perforated ears [3].

Early pre-licensure studies showed that efficacy against all-cause OM increased as complexity of disease grew (i.e. being the lowest against simple acute OM and the highest against chronic otitis with effusion) [1,4–6]. The natural progression of complexity from acute OM to recurrent/nonresponsive OM and to chronic OM

Abbreviations: OM, otitis media; PCV7, 7- valent conjugated vaccines; PCV13, 13-valent conjugated vaccines; PCV, pneumococcal conjugated vaccines; NTHi, nontypeable *H. influenzae*; MEF, middle ear fluid; VT, vaccine serotype; IRRs, incidence rate ratios; CI, confidence interval.

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with effusion is characterized by the progressively reduced role of pneumococci (especially vaccine serotypes) and increasing role of nontypeable *Haemophilus influenzae* (NTHi), mixed infections and biofilm formation [1]. However, these cases with MEF biofilm are often culture negative, in the absence of free-living planktons in the MEF [1,7].

Recently, by using post PCV7/PCV13 implementation impact studies (the "vaccine probe" approach), we demonstrated a dramatic reduction in complex OM cases caused not only by vaccine serotype (VT) *Streptococcus pneumoniae* strains but also those caused by NTHi and culture-negative cases [2,3], confirming that widespread PCV use reduces early damage of the middle ear mucosa, thus preventing complication/sequelae such as recurrence, non-responsiveness, spontaneous otorrhea and chronicity.

In southern Israel, middle ear fluid cultures are frequently obtained in severe acute OM or in complex OM, either directly from otorrhea derived from spontaneously perforated ears, or from samples obtained by tympanocentesis, when the tympanic membrane is intact at presentation. Spontaneously perforated OM in young children often represents a recurrent/chronic state with biofilm formation [8]. It is therefore possible that widespread PCV vaccination would reduce this entity through reduction of the initial step, acute OM (often caused by vaccine-type pneumococci).

Our aim was to compare the incidence dynamics of OM between episodes presenting with spontaneous otorrhea and those presenting with intact tympanic membrane, with culture obtained through tympanocentesis, following implementation of PCV7/PCV13 in Israel. We speculated that spontaneously perforated OM represents mostly complex OM, while those presenting with intact tympanic membrane (the tympanocentesis group) represent a more heterogeneous group, with a higher proportion of true acute OM.

2. Methods

This ongoing, prospective, population-based, active surveillance spanned over an 11-year period, from July 2004 through June 2016. The study was approved by the Soroka University Medical Center's Ethics Committee. Detailed methods were described previously [2,3] and will be presented here only briefly.

2.1. Setting and study population

The Soroka University Medical Center is the only hospital in southern Israel (the Negev region), with >95% of the Negev's children born and receiving medical services at this hospital [2,3]. During the study period, the average total annual births was ~15,000 [9].

Over 95% of MEF cultures from the region are sent to the Soroka University Medical Center laboratory [2,3]. OM diagnosis was made by either a pediatrician, family physician, or otolaryngologist, as previously described; cultures were obtained by tympanocentesis or pus collected from draining ears (of <7 day duration) [2,3].

The study population comprised children <3 years old with OM episodes resulting in MEF culture obtained between July 2004 and June 2016. Indications for referral, tympanocentesis or MEF culture were similar during the 11 study years. These include (but not limited to) young age (<6 months), previous OM episodes or tube insertion, high grade fever or toxic appearance and spontaneous drainage.

Demographic and clinical information was obtained from the medical charts, the child's physician, or parents, as appropriate for children with positive cultures; and included, among others, culture date, age, ethnicity, previous OM episodes and recent antibiotic treatment [2,3].

2.2. Case definition

Complex OM was defined as associated with recurrence/chronicity included a history of recurrent OM episodes (>3 episodes), antibiotic treatment in the last 30 days and previous tympanocentesis or tube insertion.

Spontaneous otorrhea included episodes with and without tympanostomy tube, as these data were not available. Notably, tympanostomy tube insertion is an uncommon practice in our region.

2.3. Bacteriology

The specimen swabs were placed in transport medium and were processed as previously described [3]. *S. pneumoniae*, NTHi, *Moraxella catarrhalis* and *Streptococcus pyogenes*, were defined as OM pathogens, and identified as previously described [2,3,10].

Pneumococcal serotyping was done by the Quellung reaction using antisera from Statens Serum Institut, Copenhagen, Denmark.

2.4. PCV7/PCV13 vaccine uptake

The inclusion of PCV7 in the national immunization plan was initiated in July 2009 with a catch-up campaign in children <2 years [2]. In November 2010, PCV13 replaced PCV7, without further catch-up.

Vaccine uptake evaluation methods were previously described [11]. By June 2011 and December 2012, ~80% and ~90%, respectively, of 7–11 month children received ≥ 2 PCV7 and/or PCV13 doses; by June 2014 and June 2016 of ~95% (both dates) received ≥ 2 PCV13 doses.

For children 24–35 months, by June 2011 and December 2012, 36% and 87%, respectively, received ≥3 PCV7 or PCV13 doses; by June 2014 and June 2016, >90% (both dates) received ≥3 PCV13 doses.

2.5. Data analysis

Otitis media episodes were separated by a 30-day interval for same pathogen or any interval for different organisms. For pneumococcus, "same organism" was defined by identical serotype; and for other pathogens, no any further intra-species characterization was done. Only one isolate was included per episode; if the same strain was isolated from both ears, only one was selected randomly [3]. A mixed infection was defined as the simultaneous presence (in the same ear or 2 different ears) of ≥2 defined pathogens.

Three sub-periods were defined, to allow better impact appreciation, both in the immediate post-PCV introduction period and long term changes following several years of vaccination; (1) pre-PCV: July 2004–June 2008; (2) PCV7: July 2009–June 2011; and (3) PCV13: July 2014–June 2016. Mean incidences during PCV7 and PCV13 periods were compared with those in the pre-PCV period.

Three age groups were defined: <12, 12–23 and 24–35 months.

Incidences were calculated using the birth cohorts born in Southern Israel, according to the Israeli Central Bureau of Statistics reports [9]. Incidence rate ratios (IRRs) and 95% confidence intervals (CIs) were calculated for specific pathogens and overall OM, and were adjusted to ethnicity (Jewish and Bedouin children) and age groups.

Data were analyzed using SPSS 22.00 software. Univariate analyses were conducted using two-tailed Chi-square test or Student-*t*-

test, where appropriate. *P* value < 0.05 was considered statistically significant.

3. Results

During the study period, 7705 OM episodes with MEF cultures were identified. Of those, 4408 (57.2%) were from tympanocentesis, 1297 (16.8%) from spontaneous otorrhea and 2000 (26.0%) from unknown source. The latter group was therefore deleted from our analysis.

In the pre-PCV period, children with spontaneous otorrhea were older (mean age 16.9 ± 8.1 *vs.* 10.9 ± 6.6 months, *P* < 0.001), with 30.1%, 49.6% and 20.3% of the children at the age of <12, 12–23 and 24–35 months, respectively, compared with. 63.1%, 32.7% and 4.2%, respectively, in the tympanocentesis group. Children with spontaneous otorrhea had higher rates of risk factors associated with recurrence/chronicity (78.3% *vs.* 70.6%, *P* = 0.002) than children with tympanocentesis. During that period, proportion of all-cause OM that was pneumococcal was 44%. The respective proportions of all-NTHi, *M. catarrhalis*, *S. pyogenes* and culture-negative OM of all-cause OM were 52%, 2%, 4% and 15%. The proportion of episodes with mixed NTHi + *S. pneumoniae* was 17%. Proportions of PCV13 serotypes (VT13) of all pneumococcal OM in children <36 months old was 84%. Similar proportions were found in the different age groups (78%–90%) and in both the spontaneous otorrhea and tympanocentesis groups.

3.1. Spontaneous otorrhea and tympanocentesis OM rate dynamics

3.1.1. PCV7 period vs. pre-PCV period

Rates of OM caused by VT13 decreased in both spontaneous otorrhea and tympanocentesis groups (due to decline in PCV7 serotypes): IRR 0.57 (0.42–0.76) and 0.49 (0.42–0.58), respectively (Table 1, Fig. 1).

Similarly, overall pneumococcal OM rates decreased in both groups; IRR 0.79 and 0.63, respectively. Rates of OM caused by non-VT13 serotypes increased in both groups: IRRs 2.17 *vs.* 1.38 in the spontaneous otorrhea and tympanocentesis groups, respectively. Thus, the declines in VT13 serotypes and overall pneumococcal OM tended to be flatter and the increase in non-VT13 serotypes tended to be steeper in the spontaneous otorrhea group, but those did not reach statistical significance (Table 1 and Fig. 1).

In contrast to episodes caused by *S. pneumoniae*, dynamics of NTHi OM differed significantly between the two groups: the rates increased non-significantly in the spontaneous otorrhea group (IRR 1.10), but significantly decreased in the tympanocentesis group (IRR 0.78). For culture-negative OM episodes, the rates increased in both spontaneous otorrhea and tympanocentesis groups; however, a significantly steeper increase was observed in the spontaneous otorrhea group (IRR 8.20) compared with the tympanocentesis group (IRR 1.69).

As a consequence of the diverging trends, the rates of all-cause OM episodes increased significantly in spontaneous otorrhea (IRR 1.56), but significantly decreased for tympanocentesis (IRR 0.89).

3.1.2. PCV13 period vs. PCV7 period

In contrast to the different dynamics observed between the 2 groups during the PCV7 period, during the PCV13 period, the rates in both spontaneous otorrhea and tympanocentesis groups showed similar declines for all subgroups. Rates of OM caused by VT13, non-VT13 and all-pneumococcal serotypes decreased by 89%, 79% and 88%, respectively, in the spontaneous otorrhea group; the respective declines in the tympanocentesis group were 94%, 55% and 80%; All NTHI OM declined by 89% and 76% in the spontaneous otorrhea and tympanocentesis groups respectively; the respective declines for culture negative OM were 69% and 47%; and for all-cause OM they were 80% and 67%.

3.1.3. PCV13 period vs. pre-PCV period (overall impact)

Rates of disease caused by VT13 in both the spontaneous otorrhea and the tympanocentesis groups decreased by >95%. Similarly, non-VT13 OM rates declined by 57% and 38%, respectively. This resulted in an overall ∼90% decline of all-pneumococcal OM in both groups. Similarly, all-NTHi OM episodes declined by 88% and 82% in the spontaneous otorrhea and the tympanocentsesis groups, respectively; In contrast to pneumococcal and NTHi OM, rates of culture-negative OM significantly increased among the spontaneous otorrhea group (IRR 2.54), but declined, among the tympanocentesis group (IRR 0.89). This was the only group for which he IRRs during the PCV13 period diverged slightly. All-cause OM rates significantly declined by 69% and 70%, in the spontaneous otorrhea and the tympanocentesis groups, respectively.

Table 1
Incidence (per 1000 population) and incidence rate ratios (IRRS; 95% CI) of complex OM in children < 36 months, Southern Israel; tympanocentesis *vs.* spontaneous otorrhea - adjusted for age & ethnicity.

OM caused by:	Spontaneous otorrhea						Tympanocentesis					
	Incidence [a] per 1000 < 36 m				IRR (95% CI)		Incidence per 1000 < 36 m				IRR (95% CI)	
	Pre-PCV period	PCV7 period	PCV13 period	PCV7 *vs.* Pre PCV	PCV13 *vs.* PCV7	PCV13 *vs.* Pre PCV	Pre-PCV period	PCV7 period	PCV13 period	PCV7 *vs.* Pre PCV	PCV13 *vs.* PCV7	PCV13 *vs.* Pre PCV
VT13	1.2 ± 0.3 (n = 196)	0.6 ± 0.0 (n = 59)	0.1 ± 0.1 (n = 4)	0.57 (0.42–0.76)	0.11 (0.05–0.25)	0.04 (0.02–0.10)	4.0 ± 0.3 (n = 694)	2.0 ± 0.6 (n = 183)	0.2 ± 0.1 (n = 13)	0.49 (0.42–0.58)	0.06 (0.04–0.11)	0.03 (0.02–0.06)
Non-VT13	0.2 ± 0.0 (n = 34)	0.4 ± 0.1 (n = 39)	0.1 ± 0.1 (n = 8)	2.17 (1.37–3.43)	0.21 (0.10–0.42)	0.43 (0.21–0.89)	0.8 ± 0.1 (n = 129)	1.1 ± 0.4 (n = 96)	0.5 ± 0.2 (n = 49)	1.38 (1.06–1.79)	0.45 (0.32–0.64)	0.62 (0.45–0.86)
All PNC	1.4 ± 0.3 (n = 233)	1.1 ± 0.2 (n = 98)	0.1 ± 0.1 (n = 13)	0.79 (0.63–1.00)	0.12 (0.07–0.21)	0.09 (0.05–0.16)	4.7 ± 0.3 (n = 824)	3.0 ± 0.8 (n = 279)	0.6 ± 0.1 (n = 63)	0.63 (0.55–0.72)	0.20 (0.15–0.26)	0.13 (0.10–0.16)
NTHi + PNC	0.7 ± 0.2 (n = 123)	0.5 ± 0.0 (n = 45)	0.0 ± 0.0 (n = 2)	0.69 (0.49–0.98)	0.02 (0.01–0.05)	0.07 (0.03–0.16)	1.6 ± 0.2 (n = 279)	1.3 ± 0.5 (n = 112)	0.2 ± 0.1 (n = 21)	0.75 (0.60–0.93)	0.17 (0.10–0.26)	0.12 (0.08–0.19)
NTHi single Cx	0.7 ± 0.2 (n = 120)	1.0 ± 0.2 (n = 89)	0.2 ± 0.1 (n = 15)	1.39 (1.06–1.83)	0.15 (0.09–0.26)	0.21 (0.12–0.36)	3.8 ± 0.6 (n = 651)	3.0 ± 1.0 (n = 276)	0.8 ± 0.0 (n = 82)	0.79 (0.69–0.91)	0.26 (0.21–0.34)	0.21 (0.16–0.26)
All NTHi	1.5 ± 0.4 (n = 259)	1.7 ± 0.2 (n = 152)	0.2 ± 0.1 (n = 19)	1.10 (0.90–1.35)	0.11 (0.07–0.18)	0.12 (0.08–0.19)	5.6 ± 0.6 (n = 959)	4.3 ± 1.6 (n = 401)	1.1 ± 0.1 (n = 107)	0.78 (0.69–0.87)	0.24 (0.19–0.29)	0.18 (0.15–0.22)
Culture negative	0.2 ± 0.2 (n = 35)	1.7 ± 0.1 (n = 152)	0.5 ± 0.3 (n = 53)	8.20 (5.68–11.85)	0.31 (0.23–0.42)	2.54 (1.65–3.89)	1.9 ± 0.5 (n = 325)	3.2 ± 0.8 (n = 295)	1.7 ± 0.1 (n = 177)	1.69 (1.44–1.97)	0.53 (0.44–0.64)	0.89 (0.74–1.07)
All-cause OM	2.7 ± 0.8 (n = 468)	4.2 ± 0.1 (n = 387)	0.9 ± 0.5 (n = 86)	1.56 (1.36–1.78)	0.20 (0.16–0.25)	0.31 (0.24–0.39)	11.0 ± 1.3 (n = 1911)	9.9 ± 2.9 (n = 912)	3.3 ± 0.0 (n = 345)	0.89 (0.82–0.96)	0.33 (0.30–0.38)	0.30 (0.27–0.33)

[a] Incidence numbers were rounded (one figure beyond the point).

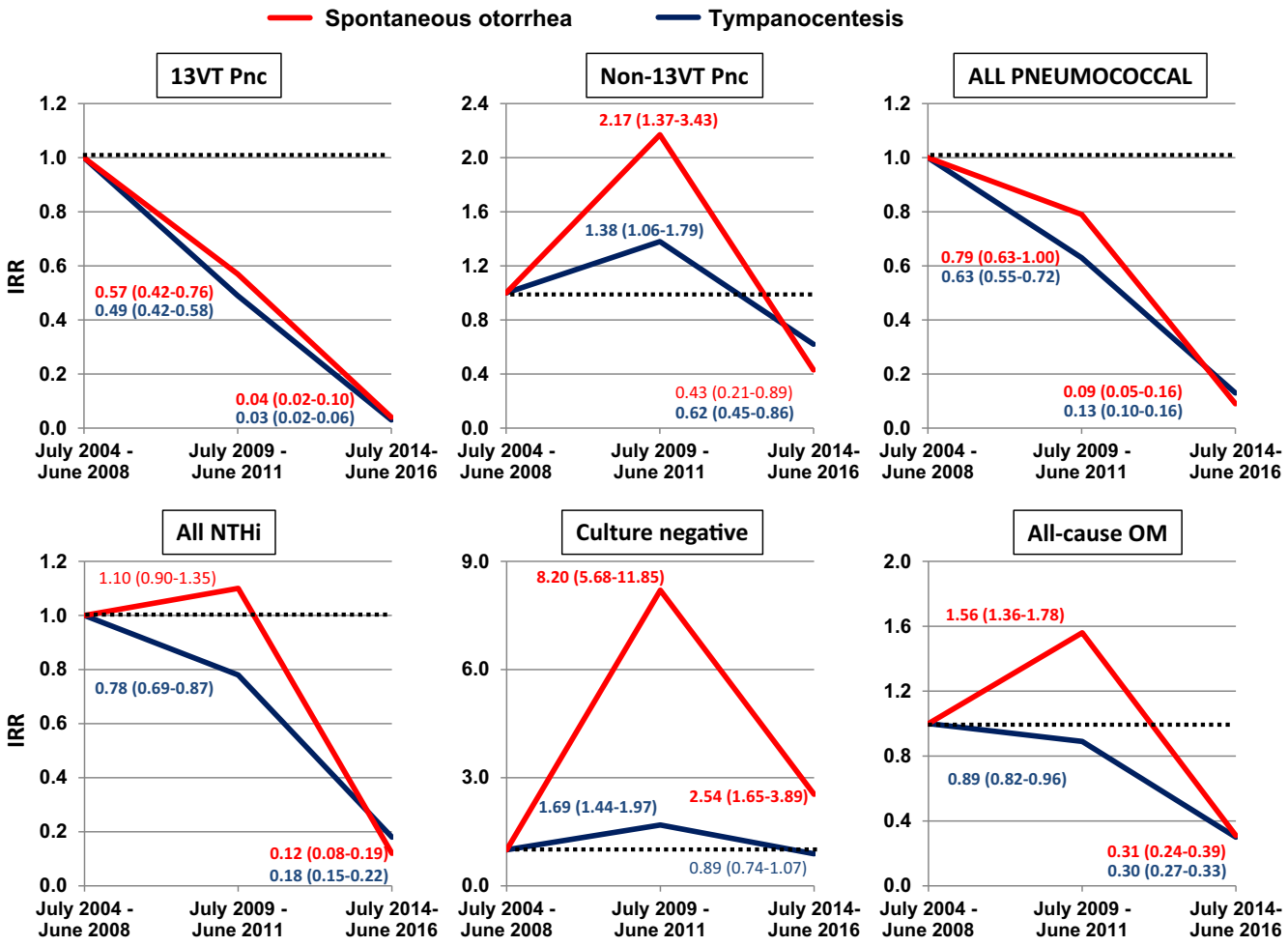

Fig. 1. Incidence rate ratios (IRRs; 95% CI) of complex OM in children <36 months old, southern Israel; tympanocentesis *vs.* spontaneous otorrhea - adjusted for age and ethnicity.

3.2. OM rates dynamics by age group

Rates of episodes in children <12 months old and 12–23 months old were similar for all subgroups to those shown in the overall group <36 months.

Beyond the differences between the spontaneous otorrhea and tympanocentesis groups, children aged 24–35 months differed from the other age groups in many regards, during the PCV7 period: (1) VT13 rates did not significantly decline in the spontaneous otorrhea group, while a significant 28% decline was observed in the tympanocentesis group; (2) non-VT13 rates increased non-significantly in both the tympanocentesis and the spontaneous otorrhea groups; (3) overall pneumococcal OM rates did not decrease in the PCV7 period, for both spontaneous otorrhea and tympanocentesis groups; (4) NTHi rates increased in the PCV7 period for the tympanocentesis group in contrast to no increase in the spontaneous otorrhea group. Furthermore, a significant decline was observed in the tympanocentesis group in children <12 months and 12–23 months.

Overall impact (comparing PCV13 period *vs.* pre-PCV period) was similar in the spontaneous otorrhea and the tympanocentesis groups in all 3 age groups for VT13 (≥90% reductions). Similarly, reductions were observed in all age groups for all-pneumococcal OM, NTHi and all-cause OM. In contrast, when comparing PCV13 period and the pre-PCV period, non-VT13 and culture-negative OM rates differed considerably among the 3 age groups: Non-VT13 rates declined in <12 months old, did not change in 12–23 months old and increased (for tympanocentesis) in 24–35 months old. Culture-negative OM rates substantially increased in all 3 age groups for the spontaneous otorrhea group but only increased significantly in the tympanocentesis group for 24–35 months old, while rates decreased significantly for <12 months old, and remained stable for the 12–23 months old.

4. Discussion

Following PCV7/PCV13 introduction, rates of OM submitted for MEF culture substantially declined for VT13, all-pneumococcal, all NTHi and all-cause OM, in both spontaneous otorrhea and tympanocentesis OM groups. These findings are important, suggesting that reduction in complex OM rates was indeed mainly driven by PCV introduction, as spontaneous otorrhea OM rates are not influenced by possible changes in indications or local practice for obtainment of MEF cultures through tympanocentesis. Furthermore, indications for referral, tympanocentesis, or MEF culture did not change during the study period in our hospital.

The current study is derived from the largest culture-proven population-based study globally, and therefore it represents a unique opportunity to study pathogen-associated OM post-PCV implementation and to compare the 2 study groups, namely children presenting with spontaneously draining ears during OM episodes *vs.* those with OM presenting with intact tympanic

membrane with MEF culture obtained through tympanocentesis. However, it is important to remember that when observing the two groups, a history of previous episodes and recurrence was frequent in both groups. Therefore, both are probably enriched with complex OM episodes.

Although by the end of the study period, both groups showed similar reductions compared to the pre-PCV period, reduction dynamics were not identical between the 2 study groups and while spontaneous otorrhea dynamics resembled that of tympanocentesis when PCV13 serotypes were examined, the dynamics of reductions were not similar for non-PCV13 serotypes, NTHi, culture-negative and all-cause OM.

Two subgroups of OM studies here deserve special attention. In the transition from acute OM to more complex episodes, such as recurrent, non-recurrent or chronic OM with effusion, the predominance of *S. pneumoniae* is replaced by the dominance of NTHi; together with increasing frequency of multiple co-pathogens and biofilm formation [1]. Furthermore, NTHi middle ear infections tend to be associated with specific non-PCV13 serotypes [12]. In addition, once biofilm is formed in many occasions the absence of free swimming planktons in the middle ear fluid results in culture-negative samples. However, more sensitive methods including PCR and biopsies reveal that unlike the common belief among many clinicians, culture-negative OM does not indicate most of the time absence of bacterial OM, but rather a complex status of OM with biofilm with higher activity of NTHi.

In this context, the higher age, frequency of previous OM episodes by history, higher rate of culture-negative episodes, delayed vaccine impact on NTHi, culture-negative and overall OM episodes compared to VT13 serotype among the spontaneous otorrhea compared to those with tympanocentesis – all strongly suggest that spontaneously perforated OM represents a more advanced chronic/recurrent stage of OM, as expected by recent insights gained in regard to complex OM evolution and its prevention by PCVs [1,2,7,13].

Non-VT13 and culture negative OM rates initially increased in the PCV7 period (with higher increase rate in spontaneous otorrhea) and then decreased in the PCV13 period in both spontaneous otorrhea and tympanocentesis OM. This suggests that, as expected, PCV initial impact in reducing complex OM rates was driven by near-elimination of VT13 disease, followed by an increase in non-vaccine serotypes and non-pneumococcal disease. The late effect of reduction in non-VT13 OM is probably attributed to reduction in early OM episodes due to both direct and indirect protection, associated with increased vaccination rates and reduced pneumococcal VT13 carriage rates and spread, thus preventing sequelae associated with non-pneumococcal pathogens [1,2].

In this regard, our results may help to understand one of the paradoxes cited in the history of PCV development. A double-blind controlled study conducted in the Netherlands in children aged 1–7 years, addressed the ability of PCV7 to reduce recurrence of OM episodes [14]. The ensuing results showed that as expected, PCV7 brought about a reduction in PCV7 serotype carriage with increasing non-PCV7 serotype carriage. However, unexpectedly, PCV7 administration significantly increased recurrent episodes by 20% with increasing culture-negative spontaneous otorrhea episodes, similar to what we observed in the spontaneous otorrhea group in our study. In view of the previous studies conducted by our group [2,3], it is suggested that initially, an increase in non-PCV13 serotype carriage could probably enhance mixed infections or biofilm formation in damaged ears, resulting in an increased rates of episodes in the vaccinated subjects.

It was expected that spontaneously perforated OM would be less impacted by PCV7 introduction, since older children with recurrent OM episodes were born before PCV7 implementation, and were therefore mostly unimmunized. In addition, children in whom chronic/recurrent OM had already being established, had probably a high proportion of mixed infections and biofilm [15–18], and thus overall, their infections were less frequently "vaccine preventable". Indirect (herd) protection is the main mechanism in preventing early acquisition of disease, especially in children too young to reach $\geq$ 2 PCV doses [3,19,20]. However, marked indirect protection can only be achieved in populations with high (>70–80%) vaccine coverage [4,21], explaining why the incidence of OM episodes among older children were only slightly reduced in the first 3 years following PCV introduction.

It is thus likely that the marked reduction of culture-negative and NTHi episodes in spontaneous otorrhea is related to the time since initiation of PCV vaccination, when children were born into an environment with a markedly reduced PCV13 serotypes circulation, preventing early OM and its resulting sequelae. The findings that spontaneous otorrhea started to decline earlier in younger children than those >12 months old support this speculation. Additional support to this hypothesis was lent by a recent Spanish study, which concluded that "PCV13 vaccination would further reduce transmission of PCV13 serotypes with special benefits for youngest children. preventing first otitis episodes and subsequent recurrences" [22].

The current study has several limitations. First, selection bias is a possibility when assessing rates of OM with MEF culture, as probably (almost) all episodes with spontaneous draining ears are being sent for culture, while for children with intact tympanic membrane, tympanocentesis is being performed selectively from children with toxic appearance, history of recurrent OM etc. Second, we do not have data regarding the rate of children with tympanostomy tube (TT), which could influence the rate of spontaneously draining ears. However, rates of TT in our populations are low, and it is unlikely that this was a major factor influencing the results. Third, it was suggested before that spontaneous perforation episodes are associated with lack of antimicrobial treatment during OM [23], suggesting that at least some spontaneous perforation episodes occurs with acute disease. Fourth, the local epidemiology presented in the current study may not reflect that in other regions (e.g. rates of *Streptococcus pyogenes* and *Moraxella catarrhalis* disease rates).

In conclusion, PCV7/PCV13 implementation reduced rates of episodes of OM submitted for culture. However, while dynamics were similar for PCV13 serotypes, dynamics of non-PCV13 serotypes, NTHi, culture-negative and all-cause OM differed between the two groups. These findings, in conjunction with the fact that children with spontaneous perforation were older and had higher proportions of risk factors for complex OM, and recent insights on complex OM evolution and its response to PCVs, strongly suggest that spontaneous otorrhea episodes represent a more advanced chronic/recurrent stage of OM.

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Conflicts of interest

Shalom Ben-Shimol has received speakers' fees and a research grant from Pfizer. **David Greenberg** has received grants from Merck Sharp & Dohme; has been a scientific consultant and a speaker for Merck Sharp & Dohme and Pfizer.

Ron Dagan has received grants/research support from Pfizer and Merck Sharp & Dohme; has been a scientific consultant for MeMed, Merck Sharp & Dohme, and Pfizer and a speaker for Pfizer.

All the other authors have no financial relationships relevant to this article to disclose.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2018.11.054.

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