Vaccine xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Pneumococcal conjugate vaccine triggers a better immune response than pneumococcal polysaccharide vaccine in patients with chronic lymphocytic leukemia A randomized study by the Swedish CLL group

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ARTICLE INFO

Article history:
Received 10 November 2017
Received in revised form 1 May 2018
Accepted 2 May 2018
Available online xxxx

Keywords:
Chronic lymphocytic leukemia (CLL)
Pneumococcal vaccine
Polysaccharide vaccine
Protein-conjugate vaccine
Immunogenicity

ABSTRACT

Aim: To determine if patients with untreated chronic lymphocytic leukemia (CLL) benefit from vaccination with a 13-valent pneumococcal conjugated vaccine (PCV13), Prevenar13®, compared to a 23-valent pneumococcal polysaccharide vaccine (PPSV23), Pneumovax®, in terms of immune response.
Background: *Streptococcus pneumoniae* causes substantial morbidity in patients with CLL, a group known to respond poorly to polysaccharide vaccines. Comparative studies with conjugated vaccines are lacking.
Methods: 128 treatment naïve CLL patients from eight hematology clinics in Sweden were randomized to vaccination with PCV13 (n = 63) or PPSV23 (n = 65) after stratification by IgG level and CLL clinical stage (Rai). Blood samples for evaluation of immune response were obtained at baseline, and at one and six months after vaccination. Analyses for each of the 12 pneumococcal serotypes common for PCV13 and PPSV23 were performed by opsonophagocytic assay (OPA) and enzyme-linked immunosorbent assay (ELISA).
Results: PCV13 elicited a superior immune response than PPSV23 in 10/12 serotypes one month after vaccination and in 5/12 serotypes six months after vaccination, measured as OPA geometric mean titers (GMTs). Geometric mean concentrations of serotype-specific IgG antibodies elicited by PCV13 as measured by ELISA, were higher than those elicited by PPSV23 in half of the common serotypes, both after one and six months. PPSV23 did not trigger a better immune response than PCV13 for any of the serotypes, regardless of analysis method or time point of analysis. Negative predictive factors for vaccination response were hypogammaglobulinemia and long disease duration. Both vaccines were well tolerated.
Conclusions: In patients with previously untreated CLL, the efficacy of PCV13 in terms of immune response is superior to PPSV23 for most serotypes common for the two vaccines. We therefore propose that PCV13 should be included in vaccination programs against *Streptococcus pneumoniae* for CLL patients and administered as early as possible during the course of the disease.

1. Introduction

Patients with chronic lymphocytic leukemia (CLL) have an increased risk of infection due to predisposing factors such as hypogammaglobulinemia, T- and NK-cell dysfunction and complement defects [1,2]. Moreover, the short- and long-term side effects of different treatment modalities, such as chemotherapy and monoclonal antibodies, add further to infection susceptibility [1–6]. Infections caused by *Streptococcus pneumoniae* are a major cause of morbidity and mortality in CLL-patients, which makes prevention essential [7–9].

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https://doi.org/10.1016/j.vaccine.2018.05.012
0264-410X/

Vaccination is a straightforward option to increase immunity and prevent infection. However, patients with CLL are known to respond inadequately to polysaccharide vaccines, which are T-cell independent, with immunization response rates in unselected CLL-patients varying from 0 to 22% in different studies [6,7,10–12]. Reasons for the low response rates are multifactorial and include impaired humoral immunity (due to a lack of functional B-cells) and defect T-cell function [11,13,14]. It has been proposed that treatment naïve patients at an early stage of disease respond better to vaccination [15].

Conjugation of the polysaccharide with a protein carrier (protein-conjugate vaccines) renders a T-cell dependent, memory inducing vaccine [8,14–16]. Pneumococcal conjugate vaccines (PCVs) were first approved for infants and are globally recommended as routine childhood immunization [16–20]. They also improve immune response to bacteria and induce an immunological memory in the elderly [14,15]. PCVs have dramatically decreased the incidence of invasive pneumococcal disease caused by the included serotypes, but a shift in the distribution of disease causing serotypes has followed [17,18,20–22]. One study, using the 7-valent PCV, suggested that a conjugate vaccine renders a higher immune response than polysaccharide vaccines in patients with CLL, reporting a response rate of 20–47%, depending on serotype [14]. However, there are no randomized studies comparing the two different types of pneumococcal vaccines in CLL patients and no consensus regarding vaccination recommendations [14,23,24].

The aim for the present study was to determine if untreated patients with CLL benefit from vaccination with a 13-valent pneumococcal conjugated vaccine (PCV13), Prevenar13®, compared with a 23-valent capsular polysaccharide vaccine (PPSV23), Pneumovax®, in terms of immune response.

2. Methods

2.1. Patient selection

Treatment naïve CLL patients, 18 years or older in all clinical stages (Rai 0-IV), from eight hematology units in Sweden, were enrolled prospectively in the study between September 2013 to June 2015. An informed consent was required from all patients and physical examination and blood chemistry was performed to evaluate WHO performance status and Rai stage. Major exclusion criteria were: previous vaccination with a pneumococcal vaccine within 5 years, symptomatic disease and/or intention to start treatment with chemotherapy and/or monoclonal antibodies within one month, other active malignancy, allergic reaction to a vaccine in the past, neutropenia (absolute neutrophil count (ANC) $<0.5 \times 10^9$/L), ongoing infection, positive Direct Antiglobulin Test (DAT) or known previous or ongoing hemolysis.

2.2. Study design

The study was designed as a two armed, randomized, non-blinded trial. After informed consent, patients were randomized at the enrollment visit using a randomized block design with equal probability to receive PCV13 or PPSV23. Because of expectation of lower immunization response in patients with low serum IgG levels and clinically advanced disease, patients were stratified by IgG levels (normal or below normal range according to the local laboratory) and CLL stage (using the Rai clinical staging system, stage 0-I-II or stage III-IV). Blood samples for immunogenicity analyses were obtained immediately before vaccination and after one and six months, respectively (Fig. 1).

The study was performed according to the International Conference on Harmonisation's Guidelines for Good Clinical Practice and the ethical principles outlined by the Declaration of Helsinki. The Ethics committee in Stockholm and the Medical Product Agency of Sweden approved the protocol. An independent contract research organization (Karolinska Trial Alliance) monitored the study. The study is registered at www.clinicaltrials.gov (NCT01892618).

2.3. Vaccines and administration

PCV13 (Prevenar13®, Pfizer; Lot Numbers F81998, G67365, J42115) contains polysaccharides of pneumococcal serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19F, 19A, and 23F individually conjugated to a nontoxic mutant form of diphtheria toxin cross-reactive material 197 (CRM197) protein, 0.85% sodium chloride, 0.02% polysorbate 80, and 0.125 mg aluminum as aluminum phosphate, per 0.5 mL dose. The vaccine contains

2.2 μg of each saccharide, except for 4.4 μg of 6B, per 0.5 mL dose, and is supplied in single-dose syringes without preservatives and stored at 2–8 °C.

PPSV23 (Pneumovax 23®, Sanofi Pasteur MSD; Lot Numbers H011754, J009828, K021211) consists of purified capsular polysaccharides from 12 of the serotypes included in PCV13 (except 6A), as well as 11 additional serotypes (2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20, 22F, and 33F). The vaccine contains 25 μg of each of the 23 purified polysaccharide serotypes per 0.5 mL dose of vaccine and contains phenol as a preservative and stored at 2–8 °C [22].

Vaccines were administered by intramuscular injection in the deltoid using 23G 25 mm needles.

2.4. Study objectives

2.4.1. Immunogenicity

Study objectives were to compare immune response after vaccination for the 12 pneumococcal serotypes common for PCV13 and PPSV23, in terms of OPA titers and serotype-specific IgG antibodies measured by ELISA.

2.4.2. Primary study objectives

Primary study objectives were to compare responses at one month after vaccination; 1. To compare OPA geometric mean titers (GMTs) for each serotype. 2. To compare the proportion of patients with positive immunological responses defined as a post vaccination OPA titer $\geq$ assay LLOQ in at least 8 of the 12 serotypes common for PCV13 and PPSV23, in the two vaccination groups, according to pre-defined response criteria. Lower limit of quantification (LLOQs) for OPA determined from assay validation experiments (serotype 1, 1:18; serotype 3, 1:12; serotype 4, 1:21; serotype 5, 1:29; serotype 6A, 1:37; serotype 6B, 1:43; serotype 7F, 1:113; serotype 9V, 1:141; serotype l4, 1:35; serotype 18C, 1:31; serotype 19A, 1:18; serotype 19F, 1:48; and serotype 23F, 1:13).

2.4.3. Secondary study objectives

Secondary study objectives were: 1. To compare immune response in terms of OPA titers six months after vaccination, using the same means of comparison as for the primary end point. 2. To compare ELISA geometric mean concentrations (GMCs) for each serotype measured one and six months after vaccination.

2.5. Laboratory methods

2.5.1. General aspects

Blood samples for immunogenicity analyses were centrifuged within 45 min from drawing of the blood and plasma was collected and frozen in labeled vials to minimum −20 °C at the local site. After study completion, all samples were sent to Pfizer's Vaccine

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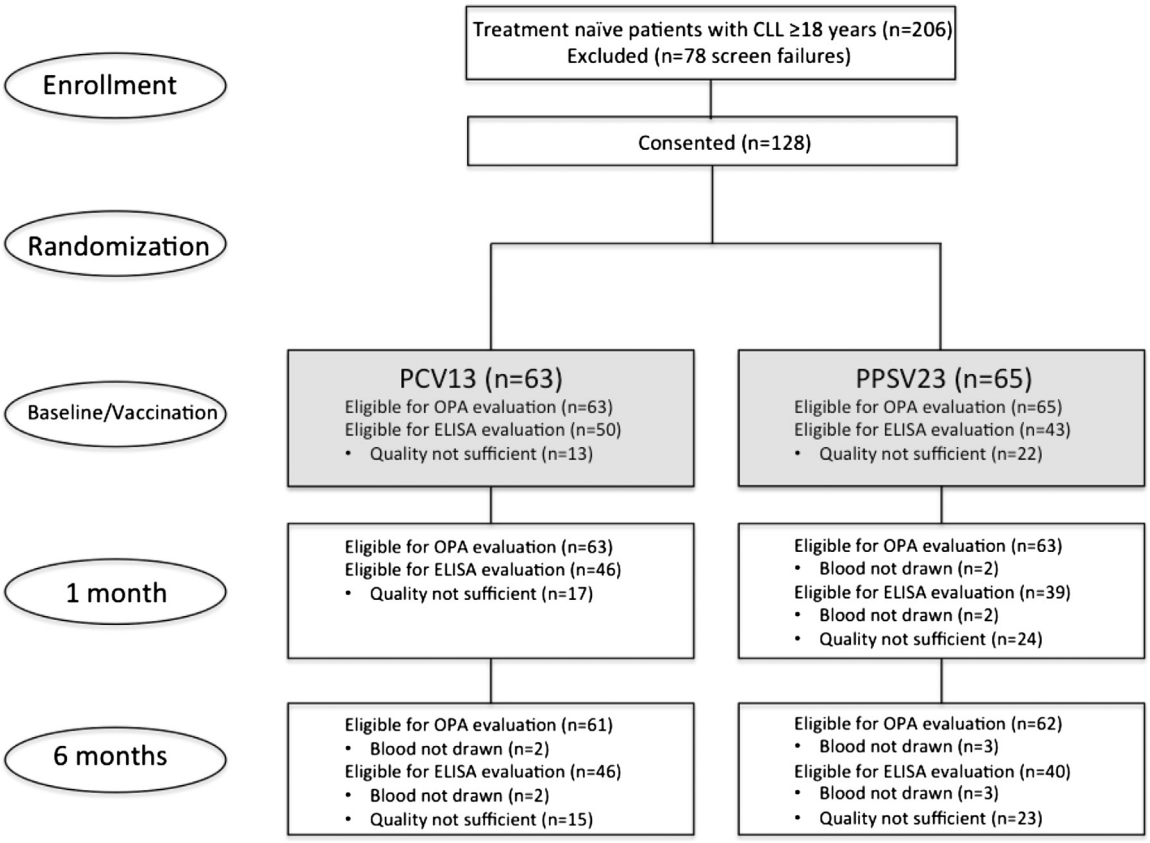

Fig. 1. Study design and disposition of subjects.

Research High Throughput Clinical Testing laboratory, Pearl River, USA for analysis using validated assays. OPA and ELISA were carried out for each of the 12 pneumococcal serotypes common to both PCV13 and PPSV23 (serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F) and for serotype 6A, found in PCV13 only. All laboratory staff was blinded to subject ID, visit ID and vaccine type administered.

2.5.2. Immunogenicity analyses

Specific functional antibacterial OPA titers were measured using validated OPA assays [25–27]. OPA titers were defined as the interpolated reciprocal serum dilution that resulted in complement-mediated killing of 50% of the assay bacteria. The lowest titer that can be determined in the assay (limit of detection [LOD]), regardless of serotype, is 1:8. However, to quantify functional antibodies with appropriate precision and accuracy, the lower limit of quantitation (LLOQ) was determined for each serotype-specific OPA assay during assay validation. Titers below the LLOQ were set to a value of 1:4 (half of the LOD). The WHO standardized enzyme-linked immunosorbant assay (ELISA) was used to measure the concentration of anti-capsular polysaccharide immunoglobulin G (IgG) elicited by the vaccine.

2.5.3. Blood chemistry analyses

Patients were monitored during the study period with blood chemistry (hemoglobin, white blood cell counts incl. differential counts, platelet counts, reticulocytes, lactate dehydrogenase (LDH), haptoglobin and DAT) before vaccination and after one and six months. Immunoglobulins (protein fraction) were analyzed by capillary electrophoresis before vaccination and after six months and IgG subclasses were analyzed by kinetic nephelometry before vaccination only. These analyses were performed locally at each hospital.

2.6. Assessment of safety

Adverse events (AEs), such as local reactions and systemic events, were monitored throughout the first month after vaccination and serious adverse events (SAEs) throughout the study period.

2.7. Statistical analyses

2.7.1. Sample size estimation

No previous clinical trials have evaluated immune response in terms of OPA GMTs (primary study objective) after pneumococcal vaccination in CLL patients. Based on a highly arbitrary expected positive immune response of 15% in PPSV23 recipients and 35% in PCV13 recipients, a 20% difference, a power of 80% (beta 0.20) and an alpha of 0.05, a sample of 145 patients would be needed. With an estimated dropout rate of 5%, 154 subjects (77 per group) should be enrolled to ensure a minimum of 145 patients evaluable for primary objectives. Due to a slow inclusion rate, a new power calculation was performed after 120 enrolled patients with the assumption of a greater difference between the groups based on new data on non-immunocompromized patients [15,16,19]. A sample size of a minimum of 120 patients was then estimated to be sufficient.

2.7.2. Immunogenicity analyses, statistical aspects

The geometric mean ratio is derived from a linear mixed model with group and time and interaction group and time as independent variables and is defined as the estimate of the interaction

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term at the specific time point (1 and 6 months respectively). The analyses were performed on the logarithm of the dependent variables, and the geometric mean ratios are the exponent of the estimated effect size measured on the log scale. The analyses of responders were performed using logistic regression models with the outcome at 1 and 6 months (performed as separate models) as dependent variables, and group and values at baseline as independent variables. All results are presented with 95% confidence intervals and p-values. Indeterminate values were not assigned a numerical value, and missing values were excluded from the immunogenicity analyses (no imputation or estimation of missing values was performed).

3. Results

3.1. Baseline characteristics

128 subjects were vaccinated from September 2013 to June 2015 (Fig. 1). In total, 126 patients concluded the study (fulfilled the one-month follow-up and were evaluable for primary endpoint), out of which 63 were vaccinated with PCV13 and 63 with PPSV23. 123 patients full-filled the six-months follow-up, but three of these patients initiated chemotherapy due to disease progression at 4, 5 and 5 months respectively after vaccination and were not evaluated for the six months immune response.

Patient characteristics are shown in Table 1. Median time from diagnosis to vaccination was 31 (range 1–248) months. Median age at the time point for vaccination was 69 (range 46–87) years. The majority of patients were in Rai stage 0-I and 35 (27%) had hypogammaglobulinemia prior to vaccination (IgG < lower normal range). Blood chemistry (hemoglobin, platelet count, LDH, reticulocytes and haptoglobin) was within normal range throughout the study.

3.2. Immune response

3.2.1. Primary objectives

One month after vaccination, OPA GMTs elicited by PCV13 were higher than those elicited by PPSV23 for 10 of the 12 common serotypes plus serotype 6A. In the other 2 common serotypes no difference was seen (Table 2, Fig. 2).

A positive immunological response, according to the predefined response criteria for OPA (≥ LLOQ), were seen in more PCV13 recipients (n = 25), than in PPSV23 recipients (n = 14) (p = 0.034), one month post vaccination (Supplementary Table 1).

3.2.2. Secondary objectives

Six months after vaccination, OPA GMTs elicited by PCV13 were higher than those elicited by PPSV23 for 5 of the 12 common serotypes plus serotype 6A. In the other 7 common serotypes no difference was seen (Table 2, Fig. 2).

A positive immunological response, according to the predefined response criteria for OPA (≥ LLOQ), were seen in more PCV13 recipients (n = 21), than in PPSV23 recipients (n = 11) (p = 0.041) (Supplementary Table 1).

In approximately one third of the intended ELISA analyses, serum sample quantity was insufficient for testing, (Fig. 1). ELISA GMCs of serotype-specific IgG antibodies in PCV13 recipients were higher than those in PPSV23 recipients for 7 of the 12 common serotypes one month after vaccination, and for 6 of the 12 common serotypes six months after vaccination. There were no significant differences in GMCs in the other common serotypes, at neither one nor six months after vaccination (Table 2).

3.2.3. Impact of hypogammaglobulinemia, time since diagnosis and Rai stage on immune response

OPA GMTs elicited by subjects with hypogammaglobulinemia (n = 35) before vaccination, were significantly lower than those elicited by patients with normal IgG values for 11/13 serotypes one

Table 1
Patient characteristics before vaccination.

Patient characteristics, *n* (%)	Total *n* = 128	PCV13 *n* = 63	PPSV23 *n* = 65
Age, median/range	69/46–87	70/46–85	68/47–87
Gender, Male/Female	65/63 (51/49)	34/29 (54/46)	31/34 (48/52)
Months from diagnosis, median/range	31/1–248	26/1–189	39/1–248
RAI stage, 0/I-II/III-IV	101/24/2 (79/19/2)	52/10/1 (83/16/2)	47/13/1 (77/21/2)
Laboratory results, mean (SD)			
Hemoglobin (g/L)	139 (14)	140 (14)	139 (14)
Platelets (10^9 cells/L)	204 (69)	208 (61)	200 (76)
WBC (10^9 cells/L)	30.1 (21.8)	30.4 (21.0)	29.9 (22.8)
ANC (10^9 cells/L)	4.7 (1.9)	4.9 (2.0)	4.4 (1.8)
Lymphocytes (10^9 cells/L)	26.4 (33.7)	25.1 (22.7)	27.7 (41.5)
Lactate dehydrogenase (μkat/L)	3.4 (3.0)	3.8 (4.2)	2.9 (0.6)
Immunoglobulin-deficiency, Yes/No/Not done (%)			
IgG deficiency	35/92/1 (27/72/1)	18/44/1 (29/70/2)	17/48/0 (26/74/0)
IgG1	22/101/5 (17/79/4)	11/51/1 (17/81/3)	11/50/4 (17/77/6)
IgG2	15/108/5 (12/14/4)	7/55/1 (11/87/2)	8/53/4 (15/82/6)
IgG3	27/96/5 (21/75/4)	14/48/1 (22/76/2)	13/48/4 (20/74/6)
IgA deficiency	38/89/1 (30/69/1)	18/44/1 (29/70/2)	20/45/0 (31/69/0)
IgM deficiency	39/88/1 (30/69/1)	14*/48/1 (22/76/2)	25*/40/0 (38/62/0)

* Difference between groups not statistically significant (p = 0.08).

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Table 2
Immunogenicity data measured as OPA-GMTs and as ELISA-GMCs (μg/ml) at baseline, at 1 month and at 6 months.

Serotype	Analysis	Baseline		1 month				6 months			
		PPSV23 GM	PCV13 GM	PPSV23 GM	PCV13 GM	GMR (95% CI)	P-value	PPSV23 GM	PCV13 GM	GMR (95% CI)	P-value
1	OPA	5.2	5.8	**10.6**	**24.0**	**2.1 (1.3–3.3)**	**0.002**	8.8	14.8	1.6 (1.0–2.5)	0.056
	ELISA	0.1	0.1	0.3	0.4	1.4 (0.9–2.1)	0.197	0.3	0.4	1.2 (0.7–1.8)	0.520
3	OPA	5.4	5.5	**9.7**	**15.5**	**1.6 (1.1–2.2)**	**0.010**	**7.5**	**10.7**	**1.4 (1.0–2.0)**	**0.052**
	ELISA	0.2	0.2	0.3	0.3	1.3 (0.9–1.8)	0.193	0.3	0.2	1.0 (0.7–1.4)	0.867
4	OPA	6.3	6.6	**27.4**	**106.3**	**3.5 (1.6–7.5)**	**0.002**	**19.9**	**52.7**	**2.5 (1.2–5.4)**	**0.019**
	ELISA	0.1	0.1	**0.2**	**0.4**	**1.7 (1.1–2.5)**	**0.015**	**0.2**	**0.4**	**1.7 (1.1–2.5)**	**0.010**
5	OPA	5.0	5.3	**9.8**	**18.1**	**1.8 (1.1–2.7)**	**0.012**	8.6	13.2	1.4 (0.9–2.2)	0.116
	ELISA	1.2	1.2	1.8	1.8	1.2 (0.9–1.6)	0.135	1.6	1.8	1.3 (1.0–1.6)	0.108
6A	OPA	7.6	6.6	**15.3**	**78.2**	**5.3 (2.8–10.1)**	**<0.001**	**15.4**	**65.2**	**4.4 (2.3–8.4)**	**<0.001**
	ELISA	1.2	0.9	**1.3**	**1.5**	**1.6 (1.2–2.1)**	**0.004**	**1.3**	**1.5**	**1.5 (1.1–2.0)**	**0.009**
6B	OPA	13.4	13.3	62.6	100.3	1.2 (0.6–2.5)	0.572	49.6	74.3	1.4 (0.6–2.8)	0.421
	ELISA	0.7	0.7	1.1	1.3	1.3 (0.9–1.8)	0.157	1.2	1.2	1.2 (0.9–1.7)	0.241
7F	OPA	12.0	10.2	**47.9**	**83.8**	**2.1 (1.1–3.9)**	**0.025**	**34.7**	**75.4**	**2.4 (1.3–4.6)**	**0.008**
	ELISA	0.5	0.4	**1.0**	**1.3**	**1.6 (1.1–2.5)**	**0.020**	1.0	1.1	1.4 (1.0–2.2)	0.085
9V	OPA	12.6	17.9	**29.9**	**111.2**	**2.9 (1.4–6.1)**	**0.005**	27.1	62.9	1.9 (0.9–4.0)	0.084
	ELISA	0.6	0.5	**0.9**	**1.4**	**1.9 (1.3–2.7)**	**0.001**	**0.9**	**1.1**	**1.6 (1.1–2.3)**	**0.011**
14	OPA	66.5	82.2	207.8	231.3	0.9 (0.5–1.7)	0.750	180.0	180.4	0.9 (0.5–1.7)	0.838
	ELISA	1.0	0.9	2.3	1.8	0.9 (0.7–1.3)	0.681	2.4	1.9	0.9 (0.7–1.3)	0.620
18C	OPA	19.9	21.4	**63.5**	**253.2**	**3.9 (2.1–7.3)**	**<0.001**	**51.0**	**128.6**	**2.8 (1.5–5.2)**	**0.001**
	ELISA	0.7	0.7	**1.2**	**2.1**	**1.7 (1.2–2.4)**	**0.004**	**1.3**	**1.7**	**1.5 (1.0–2.1)**	**0.036**
19A	OPA	17.1	18.9	**39.7**	**80.5**	**1.9 (1.2–3.0)**	**0.010**	37.9	62.0	1.6 (1.0–2.5)	0.068
	ELISA	2.2	2.0	**2.8**	**3.5**	**1.6 (1.3–2.1)**	**<0.001**	**2.7**	**3.3**	**1.4 (1.1–1.9)**	**0.005**
19F	OPA	12.1	11.5	**36.4**	**59.2**	**2.0 (1.1–3.6)**	**0.016**	29.9	37.1	1.7 (1.0–2.9)	0.075
	ELISA	0.6	0.7	**0.7**	**1.5**	**1.8 (1.3–2.5)**	**0.001**	**0.7**	**1.3**	**1.5 (1.1–2.1)**	**0.023**
23F	OPA	8.0	5.9	**12.0**	**33.2**	**3.8 (2.1–6.9)**	**<0.001**	**12.9**	**20.5**	**2.3 (1.3–4.2)**	**0.006**
	ELISA	0.8	0.7	**1.2**	**1.4**	**1.5 (1.1–2.2)**	**0.018**	**1.2**	**1.5**	**1.5 (1.1–2.2)**	**0.020**

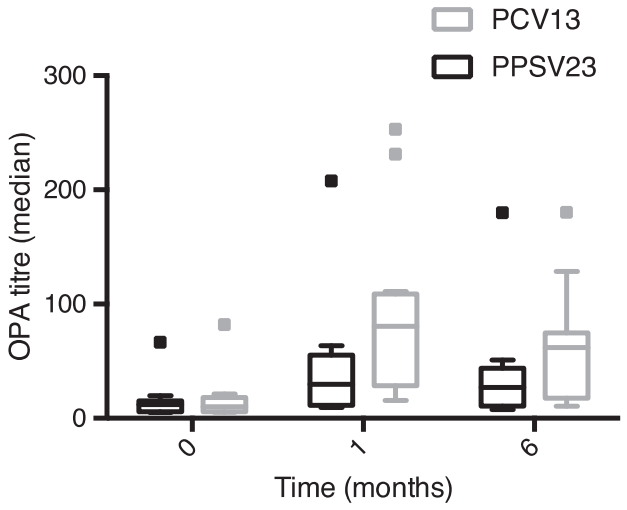

Fig. 2. Immunogenicity data measured as OPA-GMTs at baseline, at 1 month and at 6 months.

month after vaccination and for 8/13 serotypes six months after vaccination (Supplementary Table 2). There was no difference in OPA GMTs between the two vaccines in patients with hypogammaglobulinemia. In patients with an IgG value below 4.9 g/l, no positive immunological responses were seen with neither of the two vaccines (data not shown). Low values of IgM and IgA as well as low values of IgG subclasses IgG1 and IgG2 (but not IgG3) within the context of hypogammaglobulinemia, also had a negative predictive impact on vaccine response.

OPA GMTs elicited by patients with disease duration shorter than 31 months (i.e. median time from diagnosis), were higher than those elicited by patients with disease duration longer than 31 months for 13/13 serotypes one month after vaccination and for 12/13 serotypes six months after vaccination (Supplementary Table 2).

At one month after vaccination, OPA GMTs elicited by PCV13 were higher than those elicited by PPSV23 in 6/12 common serotypes plus serotype 6A in patients with short disease duration, and in 2/12 common serotypes plus serotype 6A in patients with long disease duration (data not shown).

Since only two patients were high risk (III/IV) according to the Rai staging system, analysis of the impact of Rai stage on vaccine response was not meaningful (data not shown).

3.2.4. Duration of immune response

The difference in OPA GMTs between the two vaccines was generally lower at six months than at one month after vaccination, but statistically lower for only two of the serotypes, 18C and 23 F. Measured as ELISA GMCs, the difference was significant for serotype 3 only (data not shown).

3.3. Safety analyses

In 49 (38%) patients an AE was reported. All AEs (n = 67) were grade I–II. No vaccine-related SAEs were reported.

Seven patients (6%), five in the PCV13-group and two in the PPSV23-group, had disease progression during the study period. Three patients (2%) commenced treatment before the final six months evaluation. One additional patient started intravenous gammaglobulin substitution shortly before study conclusion (data not shown).

4. Discussion

This is the first randomized trial comparing the immune response of a conjugated pneumococcal vaccine (PCV13) with a 23-valent capsular polysaccharide vaccine (PPSV23) in treatment-naïve CLL patients.

The superior efficacy of PCV13 in triggering immune response in healthy subjects, has led to its incorporation in childhood immunization programs and to a wide spread use in the clinic in elderly

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adults and other risk groups [17–19,28–31]. An improved immunogenicity against pneumococci elicited by vaccination in CLL patients could potentially decrease infectious-related morbidity. Since evaluation of clinical outcomes after vaccination is both time and resource consuming, surrogate serological markers such as OPA and/or ELISA are typically used to assess immune response in vaccination studies. In the only published study on PCV vaccination in CLL-patients, Sinisalo and co-workers showed a response rate of 20–47% for different serotypes [14]. However, their study had a limited number of patients (n = 52), and response was evaluated by ELISA only. In recent years, OPA has become the gold standard as it measures the functional capacities of vaccine-raised antibodies and determines immune response more accurately than ELISA [16,25,32].

In the present randomized study in CLL patients we found an improved immunological response, measured by OPA as well as by ELISA, with PCV13 compared to PPSV23. Most importantly, we found that OPA GMTs elicited by PCV13 were higher than those elicited by PPSV23 for 10 of the 12 common serotypes at one month after vaccination, and for 5 of the 12 common serotypes at six months after vaccination. Interestingly, a superior immune response in PCV13 recipients was seen for the 3, 7F and 19A serotypes, known to cause invasive disease globally. On the other hand, a poor immunological response, consistent with the findings by Sinisalo et al., was seen for other important serotypes, e.g. serotype 14. The difference in immunological response between the two vaccines decreased over time and was generally lower after six months than after one month.

Importantly, long duration of the CLL disease, was a negative prognostic factor for vaccination response. Although indicated in previous studies [9,10], we can here clearly show for the first time that CLL patients respond better to vaccination early in their disease, stressing that timing appears to be of major importance. Still, PCV13 was superior to PPSV23 in 2 of the 12 common serotypes also in patients with long disease duration.

A low IgG level was a negative predictive factor for vaccination response. This is however not surprising since hypogammaglobulinemia is a sign of reduced antibody production and is more common in advanced disease [6,32,33]. In our study no difference in immune response was seen between the two vaccines in patients with subnormal IgG levels, but the number of patients were low. Interestingly, no positive immunological responses were seen in patients with IgG lower than 4.9 g/l with any of the vaccines.

Our study has limitations. In around one third of the samples (the majority of these patients were from the same study site), analysis of ELISA was not possible because of insufficient serum sample quantity; probably due to lack of adherence to study protocol. Also, OPA titers were ≥LLOQ at baseline in >30% of subjects for three serotypes. This might be explained by an exposure for a specific serotype or alternatively long-lasting effects of a previous vaccination, received more than five years prior to inclusion.

The optimal vaccination strategy against pneumococci in CLL patients is largely unknown. Our results show that PCV13 elicits a better immune response than PPSV23 in most of the common serotypes, but it is important to bear in mind that PPSV23 has the advantage of covering a greater variety of pneumococcal serotypes than PCV13 [9,14]. It has been suggested that a first dose of PPSV23 can blunt the immune response to subsequent vaccinations [15,34], why an initial vaccination with PCV13 should preferably be followed by PPSV23. A revaccination study in our CLL patient cohort to examine a potential difference in immune response in PPSV23 and PCV13 recipients is highly warranted. A longer follow-up of our study is planned to determine the duration of response and possible additive effect of revaccination.

We conclude that in previously untreated CLL patients, the efficacy of PCV13 in terms of immune response is superior to PPSV23 for the majority of serotypes common for the two vaccines. PCV13 should be considered as a part of vaccination programs against *Streptococcus pneumonia* for these patients and should be administered as early as possible after CLL diagnosis.

Declaration of interest

The study was funded by governmental research funds from the Stockholm county council, Uppsala county council and supported by the national Swedish CLL-group. This study was also supported by funding and study drug (Prevenar) supply by Pfizer. Serological analyses were performed at Pfizer's Vaccine Research Clinical Testing laboratory in US. Statistical analyses were performed by Statisticon, Uppsala.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.vaccine.2018.05.012.

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