Revaccination with pneumococcal conjugate vaccine five years after primary immunization improves immunity in patients with chronic lymphocytic leukemia

Magdalena Kättström,[1] Bertil Uggla,[1] Camilla Virta,[2] Merit Melin,[2] Nina Ekström,[2] Anders Magnuson,[3] Per-Ola Andersson,[4] Ylva Hammarlund,[5] Sandra Lockmer,[6] Ingmar Nilsson,[7] Daniel Roth,[8] Magnus Svensson,[9] Tobias Tolf,[10] Eva Kimby,[6] Torbjörn Norén[11] and Simon Athlin[12]

[1]Section of Hematology, Department of Medicine, Faculty of Medicine and Health, Örebro University, Örebro, Sweden; [2]Department of Public Health, Finnish Institute for Health and Welfare, Helsinki, Finland; [3]Clinical Epidemiology and Biostatistics, School of Medical Sciences, Faculty of Medicine and Health, Örebro University, Örebro, Sweden; [4]Section of Hematology and Coagulation, Department of Specialist Medicine, Sahlgrenska University Hospital, Göteborg, Sweden; [5]Department of Medicine, Falun Hospital, Falun, Sweden; [6]Division of Hematology, Department of Medicine Huddinge, Karolinska Institute, Stockholm, Sweden; [7]Department of Medicine, Karlstad Hospital, Karlstad, Sweden; [8]Institution of Clinical Sciences, Faculty of Medicine, Lund University Hospital, Lund, Sweden; [9]Department of Medicine, Eskilstuna Hospital, Eskilstuna, Sweden; [10]Department of Medical Sciences, Section of Hematology, Uppsala University, Uppsala, Sweden; [11]Department of Laboratory Medicine, Clinical Microbiology, Faculty of Medicine and Health, Örebro University, Örebro, Sweden and [12]School of Medical Sciences, Faculty of Medicine and Health, Örebro University, Örebro, Sweden

Correspondence: M. Kättström
Magdalena.Kattstrom@regionorebrolan.se

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Abstract

Patients with chronic lymphocytic leukemia (CLL) have an impaired response to vaccination, which calls for improved vaccination strategies. This study aimed to evaluate antibody persistence 5 years after pneumococcal vaccination and response to revaccination. Seventy-four CLL patients and 31 controls, all primary immunized with 13-valent conjugated pneumococcal vaccine (PCV13) or 23-valent polysaccharide vaccine (PPSV23), were included. Antibody persistence was assessed, followed by revaccination with PCV13 and a second revaccination with PCV13 or PPSV23. Serological protection, defined as a serum serotype-specific IgG concentration ≥0.35 μg/mL for ≥70% of shared serotypes, did not differ significantly in CLL patients primary immunized with PCV13 or PPSV23 (relative risk ratio [RR]=2.7, 95% confidence interval [95% CI]: 0.5-13.1), but was lower in patients than in controls (10% *vs*. 32%; RR=0.3; 95% CI: 0.1-0.7). Following revaccination with PCV13, serological response, defined as a ≥2-fold increase for ≥70% of shared serotypes, was 24% in patients primary immunized with PCV13 compared to 12% in those primary immunized with PPSV23 (RR=2.0; 95% CI: 0.6-6.9). A second revaccination with PCV13 significantly improved serological response while PPSV23 did not further improve immunity. Our findings suggest that repeated doses of a T-cell-dependent pneumococcal vaccine improve protection in CLL patients. The study is registered at *www.clinicaltrials.gov* (NCT05316831).

Introduction

Patients with the malignant B-cell disorder chronic lymphocytic leukemia (CLL) have an increased risk of severe infections and an impaired ability to respond to vaccination.[1] Although the majority of CLL patients are asymptomatic at diagnosis and lack an indication for leukemia treatment, they often demonstrate immune dysfunctions such as hypogammaglobulinemia, T- and B-cell abnormalities and impaired complement function, which are observed already in the early stages of the disease and usually progress over time.[2-4] In addition to the immune dysfunctions, responses to vaccination are affected by various types of treatment, such as chemotherapy, CD20 monoclonal antibodies, and Bruton tyrosine kinase (BTK) inhibitors.[5-8]

Infections caused by *Streptococcus pneumoniae*, such as

pneumonia and invasive pneumococcal disease, are major causes of morbidity and mortality in CLL patients.[9-11] Several studies have shown an impaired immune response to pneumococcal vaccines in CLL patients.[6,12-16] The recommended strategy has been to primary immunize with a T-cell-dependent 13-valent conjugated pneumococcal vaccine (PCV13) followed, after 8 weeks, by a T-cell-independent polysaccharide vaccine containing 23 serotypes (PPSV23), to broaden the protection.[17] Primary immunization with a conjugated pneumococcal vaccine is supported by our previous randomized study in treatment-naïve CLL patients, which demonstrated a better immune response when PCV13 was used compared to PPSV23.[13] Vaccination with PPSV23 after PCV13, either as part of primary immunization or as revaccination after 5 years, has not shown improved immune responses in CLL patients.[15,16] To revaccinate with a conjugated vaccine has been proven to be efficient and safe in healthy elderly adults but this strategy has not been investigated in CLL patients.[18] In addition, repeated doses with conjugated vaccines have shown improved vaccine response in patients with hematologic malignancies after allogeneic stem cell transplantation,[19] which supports the use of this strategy also in other immunocompromised groups.

Our study aimed to evaluate antibody persistence 5 years after primary immunization and the antibody response to revaccination with conjugated pneumococcal vaccine in CLL patients. We hypothesized that CLL patients would benefit from this revaccination and that repeated doses of conjugated vaccine would be favorable.

Methods

Study design

In this prospective study, 74 CLL patients from our previous randomized multicenter vaccination study (2013-2016)[13] were included from October 2019 to February 2020. Thirty-one immunocompetent controls, vaccinated with PPSV23 or PCV13 between 2013-2017, were also recruited. Participants were included a median of 5 years after primary immunization and stratified into two revaccination arms based on initial PCV13 or PPSV23 vaccination (Figure 1). Detailed inclusion and exclusion criteria, as well as additional information regarding the study design, study participants and vaccines are listed in the *Online Supplementary Methods*. The study was approved by the Swedish Ethical Review Authority (2018-483, 2019-02172, 2020-00982) and the Swedish Medical Product Agency (2018/483, 2019/02172).

Immunogenicity analyses

A bead-based fluorescent multiplex immunoassay was used to quantify serum IgG against the 12 pneumococcal serotypes shared by PCV13 and PPSV23 (1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F) as described previously, with some modifications.[20] The serological assay was performed and validated by the Finnish Institute for Health and Welfare (THL), Helsinki, Finland. Serological response (SR) was defined as a ≥2-fold increase in serotype-specific IgG to ≥0.35 μg/mL and serological protection (SP) as a post-revaccination titer of ≥0.35 μg/mL. Both criteria had to be met for ≥70% of the 12 shared serotypes (9-12/12). We also evaluated a higher cut-off of ≥1.3 μg/mL, which has been proposed as a protective level for immunocompromised adults.[21] Geometric mean concentrations (GMC) and ratios (GMR) were calculated for each serotype to evaluate serotype-specific vaccine responses.

Outcomes

Primary outcomes were the proportion of CLL patients achieving SP 5 years after primary immunization with PCV13 or PPSV23 and SR 8 weeks after PCV13 revaccination. Secondary outcomes included the effect of a second revaccination with PCV13 or PPSV23 on SR. Further aims were to determine the SP rates after revaccination, assessing serotype-specific responses (GMC), examining the impact of hypogammaglobulinemia and CLL treatment on revaccination response, investigating the incidence of invasive pneumococcal disease and the prevalence of nasopharyngeal carriage.

Statistics

Baseline characteristics were compared using the Mann-Whitney test for continuous variables and χ^2 or Fisher exact test for categorical variables. Proportions of study participants with SR and SP were compared using random intercept mixed Poisson regression and presented as relative risk ratios (RR) with 95% confidence intervals (95% CI). Group, time (5 years after primary immunization, 8 weeks after first and second revaccination and 12 months after first revaccination) and their interaction (group x time) were used as fixed factors. In the mixed model analysis, missing samples were assumed as missing at random and pre-treatment adjustment (PCV13 and PPSV23) was applied when comparing CLL patients and controls. Exact McNemar and Fisher exact test were used when mixed model analysis did not converge due to sparse data.

The GMC of serotype-specific IgG were compared using a random intercept linear mixed model and presented as GMR with 95% CI. A *P* value <0.05 was regarded as statistically significant. Analyses were performed with SPSS version 29 and STATA release 17.

Results

Baseline characteristics

The baseline characteristics of CLL patients and controls are presented in Table 1. At inclusion, hypogammaglobulinemia was observed in 18 (25%) CLL patients. Sixty-one

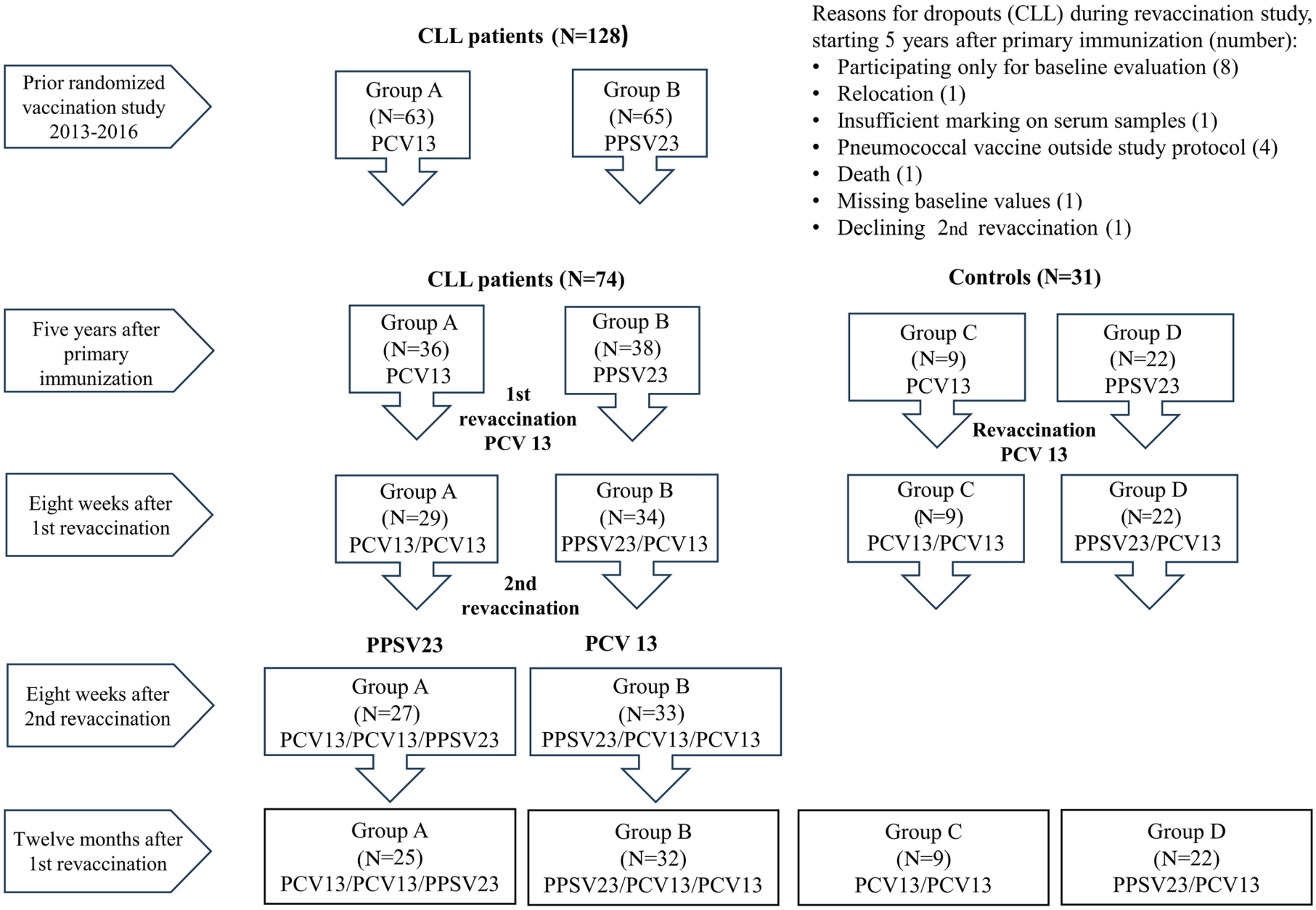

Figure 1. Study design, study population and revaccination strategy among patients with chronic lymphocytic leukemia and immunocompetent controls. CLL: chronic lymphocytic leukemia; PCV13: 13-valent conjugated pneumococcal vaccine; PPSV23: 23-valent polysaccharide vaccine.

patients (82%) were still treatment-naïve, five (7%) were off treatment in remission and seven (9%) had ongoing treatment with BTK inhibitors or had received anti-CD20 antibodies within the preceding 12 months. No significant differences in baseline characteristics were observed within the CLL cohort (group A [PCV13/PCV13] *vs*. group B ([PPSV23/PCV13]). Baseline characteristics were similar between CLL patients and controls regarding age, gender and time since immunization but differed regarding lymphocyte counts and immunoglobulin levels.

Long-term antibody persistence after primary immunization with PCV13 or PPSV23

Five years after primary immunization with PCV13 or PPSV23, the proportions of CLL patients still maintaining SP with the cut-off ≥0.35 μg/mL did not differ significantly between group A and B (14% *vs*. 5%, respectively; RR=2.7 [95% CI: 0.5-13.1]; *P*=0.23) (Table 2, Figure 2). CLL patients had a lower proportion of SP compared to controls (10% *vs*. 32%; *P*=0.006) (Table 3, Figure 3). None of the CLL patients, but 2/31 of controls (both previously immunized with PCV13) reached SP with a cut-off ≥1.3 μg/mL.

Serotype-specific IgG GMC did not differ significantly between group A and group B (*Online Supplementary Table S1*) but when comparing all CLL patients with controls, GMC were higher for 8/12 serotypes in the control group (*Online Supplementary Table S2*).

Immunity 8 weeks after first revaccination with PCV13

Following revaccination with PCV13, 24% of CLL patients in group A (PCV13/PCV13) obtained SR compared to 12% in group B (PPSV23/PCV13), however the difference was not statistically significant (RR=2.0, 95% CI: 0.6-6.9; *P*=0.25) (Table 2, Figure 2). Lower rates of SR were observed in CLL patients than in controls (18% *vs*. 42 %, RR=0.4; 95% CI: 0.2-0.7; *P*=0.04) (Table 3, Figure 3). The proportion of CLL patients and controls with SP ≥0.35 μg/mL increased significantly in all groups after revaccination (Table 3). Using the cut-off ≥1.3 μg/mL, the proportion of CLL patients increased in group A and both control groups, but not in

Table 1. Characteristics of chronic lymphocytic leukemia patients and controls.

Characteristics	CLL patients					Controls					*P* CLL *vs.* controls
	Group A, N=36		Group B, N=38		*P*	Group C, N=9		Group D, N=22		*P*	
	N		N			N		N			
Age in years, median (IQR)	36	75.5 (71.0-81.5)	38	73.0 (68.0-76.0)	0.08	9	69.7 (66.0-73.0)	22	77.5 (75.0-81.0)	0.004	0.20
Female gender, N (%)	36	19 (52.8)	38	16 (42.1)	0.36	9	5 (55.6)	22	16 (72.7)	0.42	0.06
Lymphocyte count x 10^9/L, median (IQR)	35	20.5 (5.6-45.9)	36	13.1 (5.6-21.9)	0.28	9	1.8 (1.2-2.2)	22	1.7 (1.6-2.0)	0.97	<0.001
Time since immunization in months, median (IQR)	36	66.0 (58.5-72.5)	38	62.5 (56.0-68.0)	0.13	9	56.0 (47.0-102.0)	22	63.5 (47.0-82.0)	0.69	0.92
Time since diagnosis in months, median (IQR)	36	83 (66-126)	38	105 (63-154)	0.51	NA					
Hypogammaglobulinemia, N (%)	36	10 (27.8)	37	8 (21.6)	0.54	9	1 (11.1)	22	0 (0.0)	0.29	0.01
Total IgG, g/L, median (IQR)	36	8.0 (6.4-10.6)	37	9.0 (6.9-11.2)	0.61	9	10.0 (9.4-13.2)	22	11.9 (9.6-14.3)	0.38	<0.001
Total IgM, g/L, median (IQR)	36	0.60 (0.27-0.83)	37	0.37 (0.22-0.61)	0.08	9	0.98 (0.57-1.30)	22	0.91 (0.56-1.40)	0.84	<0.001
Total IgA, g/L, median (IQR)	36	1.6 (0.7-2.2)	37	1.5 (0.8-2.2)	0.87	9	2.8 (2.0-3.0)	22	2.1 (1.7-4.6)	0.74	<0.001
IgG2, g/L, median (IQR)	35	2.3 (1.6-3.4)	32	2.3 (1.6-3.5)	0.80	8	2.2 (1.3-3.7)	21	3.4 (2.0-3.7)	0.24	0.14
Low IgG2, N (%)	35	6 (17.1)	32	6 (18.8)	0.86	8	3 (37.5)	21	2 (9.5)	0.11	0.94
Treatment status, N (%)	36	-	37	-	>0.99	NA					
Untreated	-	31 (86.1)	-	30 (81.1)	-						
Treated in remission	-	2 (5.6)	-	3 (8.1)	-						
Ongoing treatment/within 12 months	-	3 (8.3)	-	4 (10.8)	-						

CLL: chronic lymphocytic leukemia; IQR: 25th - 75th percentile; group A: PCV13/PCV13/PPSV13; group B: PPSV23/PCV13/PCV13; group C PCV13/PCV13; group D PPSV23/PCV13; NA: not applicable. *P* values were calculated with a Mann-Whitney test for continuous variables and χ^2 or Fisher exact test when appropriate for categorical variables.

group B (primary immunized with PPSV23) (Table 2, Figure 2, *Online Supplementary Table S3*).

Serotype-specific GMC increased significantly after revaccination for all serotypes in both CLL patients and controls (*Online Supplementary Tables S1, S2* and *S4*). GMC were significantly higher in 4/12 serotypes in group A than in group B (*Online Supplementary Table S1*). For controls, GMC were significantly higher for 5/12 serotypes in group C (PCV13/PCV13) compared to group D (PPSV23/PCV13) (*Online Supplementary Table S4*). GMC of IgG for all 12 serotypes were significantly higher in the controls than in CLL patients (*Online Supplementary Table S2*).

Immunity 8 weeks after second revaccination with PCV13 or PPSV23

Following a second revaccination with PCV13 (group B: PPSV23/PCV13/PCV13), the proportion with SR increased significantly, from 12% to 30% (*P*=0.017) (Table 2, Figure 2). Additionally, the proportion of patients with SP ≥0.35 μg/mL increased significantly from 27% to 49% (*P*<0.01) but no significant change was observed at the cut-off level ≥1.3 μg/mL. Following a second revaccination with PPSV23 (group A: PCV13/PCV13/PPSV23) the proportion of CLL patients with SR or SP did not increase further (Table 2, Figure 2).

Serotype-specific IgG GMC increased significantly after the second revaccination for 8/12 serotypes in group B but no further increase was seen in group A, thus decreasing the difference seen between the groups after first revaccination (from higher GMC for 4/12 to 1/12 serotypes, *Online Supplementary Table S1*).

Immunity 12 months after the first revaccination

The proportion of CLL patients with SP did not decrease significantly at any cut-off level 12 months after the first revaccination (Table 2). A significant difference remained between the CLL patients and controls regarding SP ≥0.35 μg/mL (40% *vs*. 71%; *P*=0.002), but not at the cut-off level ≥1.3 μg/mL or proportion with remaining SR (Table 3). Proportions of CLL patients with remaining SR decreased significantly in group B but not in group A (from 30% to 13%, *P*=0.021, and 30% to 20 %, *P*=0.14, respectively) (Table 2).

In both CLL patients and controls, the proportions of patients with SP were higher 12 months after revaccination compared to before the first revaccination (*P*<0.001 and *P*=0.004, respectively) (Table 3).

Serotype-specific IgG GMC decreased significantly during

Table 2. Comparison of serological protection and serological response between and within groups A and B of patients with chronic lymphocytic leukemia before revaccination (5 years after primary immunization), 8 weeks after revaccination 1, 8 weeks after revaccination 2 and 12 months after revaccination 1 using a mixed model analysis.

Between groups

Serological status	Before revaccination				8 weeks after revaccination 1				8 weeks after revaccination 2				12 months after revaccination 1			
	Group A N=36	Group B N=38	Group A *vs.* B		Group A N=29	Group B N=34	Group A *vs.* B		Group A N=27	Group B N=33	Group A *vs.* B		Group A N=25	Group B N=32	Group A *vs.* B	
	N (%)	N (%)	RR (95% CI)	*P*	N (%)	N (%)	RR (95% CI)	*P*	N (%)	N (%)	RR (95% CI)	*P*	N (%)	N (%)	RR (95% CI)	*P*
SP ≥0.35 μg/mL[a]	5 (13.9)	2 (5.3)	2.7 (0.5-13.1)	0.23	15 (51.7)	9 (26.5)	2.0 (0.9-4.0)	0.052	15 (55.6)	16 (48.5)	1.1 (0.7-1.9)	0.65	11 (44.0)	12 (37.5)	1.2 (0.6-2.2)	0.68
SP ≥1.3 μg/mL[b]	0 (0.0)	0 (0.0)	NA	NA	7 (24.1)	4 (11.8)	2.6 (0.6-11.3)	0.20	6 (22.2)	3 (9.1)	2.8 (0.6-13.4)	0.21	4 (16.0)	3 (9.4)	2.1 (0.4-11.0)	0.39
SR 2-fold increase[c]	NA	NA	NA	NA	7 (24.1)	4 (11.8)	2.0 (0.6-6.9)	0.25	8 (29.6)	10 (30.3)	1.0 (0.4-2.4)	0.92	5 (20.0)	4 (12.5)	1.6 (0.4-5.8)	0.46

Within groups

Serological status	Change 8 weeks after revaccination 1 *vs.* before revaccination						Change 8 weeks after revaccination 2 *vs.* before revaccination					
	Group A		Group B		Group A *vs.* B		Group A		Group B		Group A *vs.* B	
	RR (95% CI)	*P*	RR (95% CI)	*P*	RR (95% CI)	*P*	RR (95% CI)	*P*	RR (95% CI)	*P*	RR (95% CI)	*P*
SP ≥0.35 μg/mL[a]	3.7 (1.7-8.1)	<0.001	5.0 (1.4-17.3)	0.011	0.7 (0.2-3.2)	0.69	3.9 (1.8-8.5)	<0.001	9.3 (2.5-34.6)	<0.001	0.4 (0.1-2.0)	0.27
SP ≥1.3 μg/mL[b]	NA	0.016[d]	NA	0.12[e]	NA	NA	NA	0.031[f]	NA	0.25[g]	NA	NA
SR 2-fold increase	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Serological status	Change 8 weeks after revaccination 2 *vs.* 8 weeks after revaccination 1						Change 12 months after revaccination 1 *vs.* before revaccination					
	Group A		Group B		Group A *vs.* B		Group A		Group B		Group A *vs.* B	
	RR (95% CI)	*P*	RR (95% CI)	*P*	RR (95% CI)	*P*	RR (95% CI)	*P*	RR (95% CI)	*P*	RR (95% CI)	*P*
SP ≥0.35 μg/mL[a]	1.0 (0.9-1.3)	0.62	1.8 (1.1-2.9)	0.010	0.6 (0.3-0.9)	0.029	3.1 (1.5-6.5)	0.003	7.2 (2.0-26.0)	0.003	0.4 (0.1-1.9)	0.27
SP ≥1.3 μg/mL[b]	0.9 (0.6-1.2)	0.43	0.8 (0.6-1.2)	0.36	1.0 (0.6-1.7)	0.82	NA	0.12[h]	NA	0.25[i]	NA	NA
SR 2-fold increase	1.2 (0.7-1.9)	0.45	2.6 (1.2-5.5)	0.017	0.5 (0.2-1.1)	0.097	NA	-	NA	-	NA	NA

Serological status	Change 12 months after revaccination 1 *vs.* 8 weeks after revaccination 2					
	Group A		Group B		Group A *vs.* B	
	RR (95% CI)	*P*	RR (95% CI)	*P*	RR (95% CI)	*P*
SP ≥0.35 μg/mL[a]	0.8 (0.6-1.1)	0.10	0.8 (0.6-1.0)	0.056	1.0 (0.7-1.5)	0.94
SP ≥1.3 μg/mL[b]	0.8 (0.5-1.2)	0.25	1.0 (0.4-2.6)	0.97	0.7 (0.3-2.1)	0.59
SR 2-fold increase	0.7 (0.4-1.1)	0.14	0.4 (0.2-0.9)	0.021	1.7 (0.7-4.2)	0.25

[a]Serological protection defined as ≥0.35 μg/mL for at least nine (70 %) of the 12 serotypes. [b]Serological protection defined as ≥1.3 μg/mL for at least nine (70 %) of the 12 serotypes. [c]Serological response defined as a 2-fold increase above IgG levels ≥0.35 μg/mL in at least nine (70%) of the 12 serotypes compared to baseline. [d,e,f,g,h,i]Exact McNemar test when the mixed model analysis did not converge due to sparse data; [d]7 of 29 *vs.* 0 of 29, [e]4 of 34 *vs.* 0 of 34, [f]6 of 27 *vs.* 0 of 27, [g]3 of 33 *vs.* 0 of 33, [h]4 of 25 *vs.* 0 of 25, [i]3 of 32 *vs.* 0 of 32. Group A: patients with chronic lymphocytic leukemia vaccinated with PCV13/PCV13/PPSV13; Group B: patients with chronic lymphocytic leukemia vaccinated with PPSV23/PCV13/PCV13. RR: relative risk ratio; 95% CI: 95% confidence interval; SP: serological protection; SR: serological response; NA: not applicable.

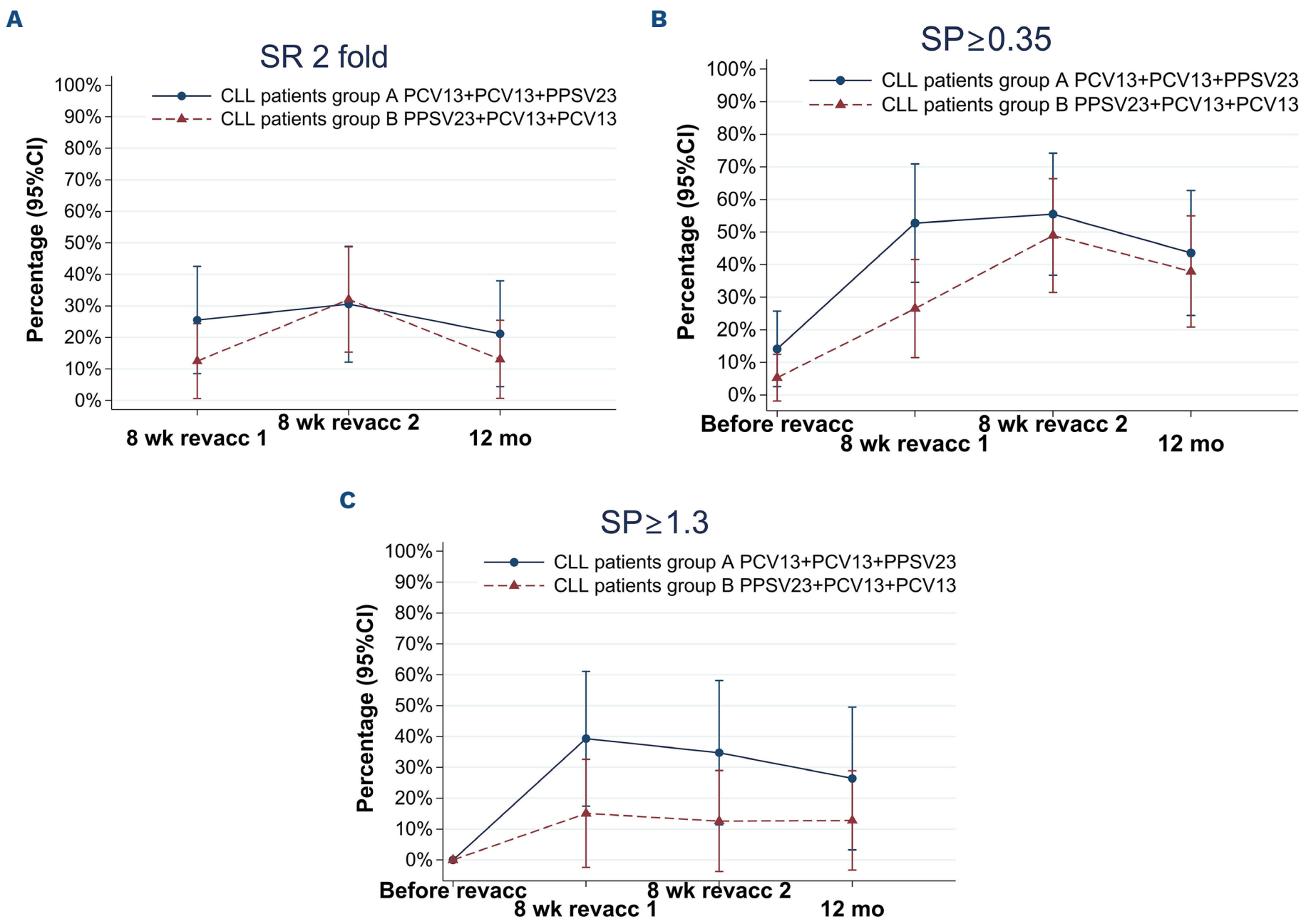

Figure 2. Serological response and protection in patients with chronic lymphocytic leukemia. (A) Serological response (SR) in group A and group B after revaccination 1, after revaccination 2 and 12 months after revaccination 1. SR defined as a 2-fold increase above IgG levels ≥0.35 μg/mL in at least nine (70%) of the 12 serotypes compared to baseline. (B) Serological protection (SP) in group A and group B before revaccination (5 years after primary immunization), after revaccination 1, after revaccination 2 and 12 months after revaccination 1. SP defined as ≥0.35 μg/mL for at least nine (70%) of the 12 serotypes. (C) SP in group A and group B before revaccination (5 years after primary immunization), after revaccination 1, after revaccination 2 and 12 months after revaccination 1. SP defined as ≥1.3 μg/mL for at least nine (70 %) of the 12 serotypes. 95% CI: 95% confidence interval; CLL: chronic lymphocytic leukemia; PCV13: 13-valent conjugated pneumococcal vaccine; PPSV23: 23-valent polysaccharide vaccine; wk: weeks; mo: months; revacc: revaccination.

the 12 months after the first revaccination (10 months after the second revaccination) and were lower for 11/12 serotypes in group A and for 6/12 in group B. However, GMC were higher for all measured serotypes in both groups compared to before the first revaccination (*Online Supplementary Table S1*). GMC were higher for 10/12 serotypes in controls compared to CLL patients and the decline of antibody concentrations was less pronounced (*Online Supplementary Table S2*).

Impact of hypogammaglobulinemia and leukemia-specific treatment on immunity after pneumococcal revaccination

Hypogammaglobulinemia was observed in 24% of the CLL patients, equally distributed between groups A and B (Table 1). Five years after the primary immunization, serotype-specific GMC were significantly higher for four of the measured serotypes in patients without hypogammaglobulinemia, both adjusted and unadjusted to previous vaccination strategy (*Online Supplementary Table S5*). After both vaccinations, the difference increased further with significantly higher GMC in the group without hypogammaglobulinemia for 11 serotypes after first revaccination and for all measured serotypes after second revaccination. The difference remained until the 12-month follow-up. None of the patients with ongoing treatment with the BTK inhibitor ibrutinib (N=3) or ongoing or recent treatment (within 12 months) with bendamustine and rituximab (N=3) reached SR at any timepoint. Only one patient, who discontinued fludarabine, cyclophosphamide and

Table 3. Comparison of serological protection and serological response between and within patients with chronic lymphocytic leukemia (groups A and B) and controls (groups C and D) before revaccination, 8 weeks after revaccination and 12 months after revaccination using a mixed model analysis.

Between groups

Serological status	Before revaccination				8 weeks after revaccination 1				8 weeks after revaccination 1			
	CLL patients N=74	Controls N=31	CLL patients *vs.* Controls		CLL patients N=63	Controls N=31	CLL patients *vs.* Controls		CLL patients N=57	Controls N=31	CLL patients *vs.* Controls	
	N (%)	N (%)	RR (95% CI)[d]	*P*	N (%)	N (%)	RR (95% CI)[d]	*P*	N (%)	N (%)	RR (95% CI)[d]	*P*
SP ≥0.35 μg/mL[a]	7 (9.5)	10 (32.3)	0.3 (0.1-0.7)	0.006	24 (38.1)	25 (80.6)	0.5 (0.3-0.7)	<0.001	23 (40.3)	22 (71.0)	0.6 (0.4-0.8)	0.004
SP ≥1.3 μg/mL[b]	0 (0.0)	2 (6.4)	NA	0.085[e]	11 (17.5)	18 (58.1)	0.3 (0.1-0.5)	<0.001	7 (12.3)	5 (16.1)	0.7 (0.2-2.0)	0.46
SR 2-fold increase[c]	NA	NA	NA	NA	11 (17.5)	13 (41.9)	0.4 (0.2-0.7)	0.004	9 (15.8)	6 (19.3)	0.7 (0.3-1.8)	0.47

Within groups

Serological status	Change 8 weeks after revaccination *vs.* before revaccination						Change 12 months after revaccination *vs.* before revaccination					
	CLL patients		Controls		CLL patients *vs.* Controls		Patients		Controls		CLL patients *vs.* Controls	
	RR (95% CI)	*P*	RR (95% CI)	*P*	RR (95% CI)	*P*	RR (95% CI)	*P*	RR (95% CI)	*P*	RR (95% CI)	*P*
SP ≥0.35 μg/mL[a]	4.6 (2.1-10.1)	<0.001	2.7 (1.4-5.0)	0.002	1.7 (0.7-3.8)	0.20	5.6 (2.5-12.7)	<0.001	2.6 (1.3-5.0)	0.004	2.1 (0.9-4.9)	0.067
SP ≥1.3 μg/mL[b]	NA	<0.001[f]	9.0 (2.4-33.4)	<0.001	NA	NA	NA	0.016[g]	NA	0.25[h]	NA	NA
SR 2-fold increase	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Serological status	Change 12 months *vs.* 8 weeks after revaccination 1					
	CLL patients		Controls		CLL patients *vs.* Controls	
	RR (95% CI)[d]	*P*	RR (95% CI)[d]	*P*	RR (95% CI)[d]	*P*
SP ≥ 0.35 μg/mL[a]	1.2 (0.9-1.7)	0.27	1.0 (0.8-1.2)	0.72	1.3 (0.9-1.8)	0.15
SP ≥ 1.3 μg/mL[b]	0.6 (0.2-1.6)	0.34	0.3 (0.1-0.6)	0.002	2.5 (0.9-6.5)	0.060
SR 2-fold increase[c]	0.7 (0.3-1.8)	0.49	0.4 (0.2-0.8)	0.014	1.9 (0.8-4.2)	0.13

[a]Serological protection defined as ≥0.35 μg/mL for at least nine (70%) of the 12 serotypes. [b]Serological protection defined as ≥1.3 μg/mL for at least nine (70%) of the 12 serotypes. [c]Serological response defined as a 2-fold increase above IgG levels ≥0.35 μg/mL for at least nine (70%) of the 12 serotypes compared to baseline. [d]Adjusted for pre-treatment (PCV13 or PPSV23). In the group of patients with chronic lymphocytic leukemia, 36 of 74 (48.6%) had PCV13 as pre-treatment, whereas in the control group 9 of 31 (29.0%) had PCV13 as pre-treatment. [e]Fisher exact test when mixed model analysis did not converge due to sparse data. [f,g,h]Exact McNemar test when mixed model analysis did not converge due to sparse data. [f]0 of 63 *vs.* 11 of 63; [g]0 of 57 *vs.* 7 of 57; [h]2 of 31 *vs.* 5 of 31. RR: relative risk ratio; 95% CI: 95% confidence interval; SP: serological protection; SR: serological response; NA: not applicable.

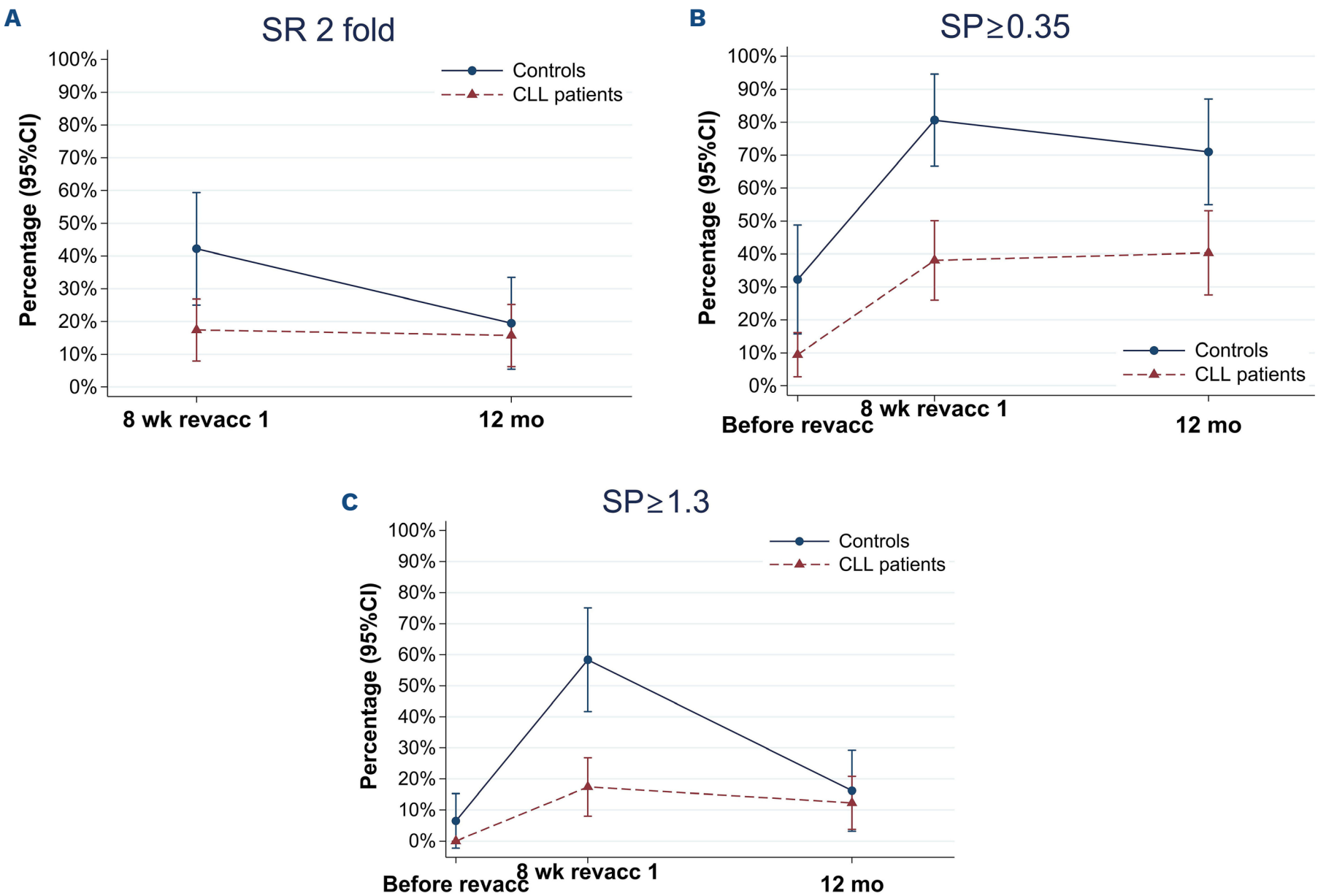

Figure 3. Serological response and protection in patients with chronic lymphocytic leukemia and controls. (A) Serological response (SR) in patients with chronic lymphocytic leukemia (CLL) and controls after revaccination 1 and 12 months after revaccination. SR defined as a 2-fold increase above IgG levels ≥0.35 μg/mL for at least nine (70%) of the 12 serotypes compared to baseline. (B) Serological protection (SP) in CLL patients and controls 5 years after primary immunization, after revaccination 1 and 12 months after revaccination. SP defined as ≥0.35 μg/mL for at least nine (70%) of the 12 serotypes. (C) SP in CLL patients and controls 5 years after primary immunization, after revaccination 1 and 12 months after revaccination. SP defined as ≥1.3 μg/mL for at least nine (70%) of the 12 serotypes. 95% CI: 95% confidence interval; PCV13: 13-valent conjugated pneumococcal vaccine; PPSV23: 23-valent polysaccharide vaccine; wk: weeks; mo: months; revacc: revaccination.

rituximab 1 month prior to inclusion in the study, presented with SP with a cut-off ≥0.35 μg/mL already at baseline and remained positive during the study period (*data not shown*).

Pneumococcal carriage and invasive pneumococcal disease

One of the study participants (in group A) was an asymptomatic pneumococcal carrier at baseline and one (in group B) 8 weeks after second revaccination. No episodes of invasive pneumococcal disease were reported in the case report forms since the start of the first vaccination study in 2013.

Safety of revaccination

Revaccinations with PCV13 and PPSV23 were well tolerated. Only expected and grade I-II adverse events were reported and were similar between groups; these events included local reaction (tenderness and pain) at the injection site, fever, fatigue and headache. No serious adverse events were observed.

Discussion

This is, to our knowledge, the first study evaluating serological responses after revaccination with conjugated pneumococcal vaccines in CLL patients. Previous studies have demonstrated an increased risk of invasive pneumococcal disease and an impaired antibody response after pneumococcal vaccination in CLL patients.[6,8-10,13-16] Therefore, optimizing the vaccination strategy to enhance protection from severe pneumococcal disease is highly warranted. In

our study, CLL patients demonstrated impaired long-term antibody persistence 5 years after primary immunization with PCV13 or PPSV23 compared to controls, but following revaccination with PCV13, the immunity improved in all participants, including CLL patients. The strategy to revaccinate CLL patients with two consecutive doses of PCV13, 8 weeks apart, was safe and improved the response further. When Lindström and coworkers previously investigated serological persistence 5 years after PCV7 vaccination in 24 CLL patients, the median antibody concentrations had declined 50-75%, depending on serotype, but more than half of the CLL patients showed remaining protective levels for 4/7 serotypes.[22] In our study, only 14% of CLL patients demonstrated SP 5 years after primary immunization with PCV13, while those immunized with PPSV23 had even lower protection (5%). Controls also showed a decline of antibody concentration over time, as only one-third had persistent antibody concentrations above SP levels 5 years after the primary vaccination. After the first revaccination, induced antibody concentrations started to decline already after 12 months in both CLL patients and controls, which may indicate a need for boosting immunity repetitively to maintain protection. Similarly, declining serotype-specific IgG concentrations have been shown in previous studies of healthy adults, measured both 12 months and 5 years after primary immunization with PCV13.[23,24]

Even though only 18% of CLL patients reached the criteria for SR after revaccination with PCV13 compared to 42% of the controls (P=0.004), improved immunity measured as increased serotype-specific GMC and proportions of patients with SP was notable. The proportions of patients with SR and SP after revaccination were higher among CLL patients primary immunized with PCV13 as compared to PPSV23 but the differences were not statistically significant (P=0.25 and P=0.052, respectively), probably due to lack of statistical power. Nevertheless, these are important results since studies evaluating revaccination strategies with conjugated pneumococcal vaccines are scarce. A previous study on revaccination with PCV20 in healthy adults previously vaccinated with PPSV23, PCV13 or PCV13/PPSV23 showed that the strategy was safe and elicited robust immune responses, regardless of the type of primary immunization.[18] Administration of two consecutive doses of PCV13 as a revaccination strategy was beneficial in our study, increasing the proportion of CLL patients with SR from 12% after the first revaccination to 30% after the second revaccination (P=0.017). This finding supports the use of repeated doses of conjugated pneumococcal vaccine in CLL patients. The stimulation of $CD4^+$ T cells and establishment of immunological memory with a conjugated vaccine may provide conditions for a potential booster effect after revaccination. In a previous study, we demonstrated that repeated doses of PCV13 led to early plasmablast expansion and an increase in switched memory cells.[25] This supports the present serological results.

There is evidence for repeated doses of conjugated pneumococcal vaccines as primary immunization for infants and patients with hematologic malignancies previously treated with allogeneic stem cell transplantation.[26,27] This strategy has shown to induce protective antibody levels, with some serotype variability, in 40% of allogeneic stem cell transplant patients a decade after primary immunization[28] which supports the use of repeated doses of conjugated pneumococcal vaccines also in other immunocompromised groups. Our study results demonstrate the potential benefit of repeated conjugate vaccinations in CLL patients and, considering the long-term effects previously observed in allogeneic stem cell transplant recipients, it is reasonable to expect that CLL patients might benefit from repeated vaccinations as a primary immunization strategy as well as periodic booster vaccinations.

Adding PPSV23 to PCV13, as recommended in guidelines,[17] did not enhance immunity in CLL patients in our study. This is in line with other study results showing that administration of PPSV23 after a conjugated pneumococcal vaccine did not improve immunity in CLL patients, either at 8 weeks or 5 years after primary immunization,[15,16] challenging the benefit of this recommendation. Another reason for adding PPSV23 is to broaden the serotype protection, including potential invasive pneumococcal disease serotypes not covered by PCV13. As a result of the introduction of PCV in national immunization programs a shift in pneumococcal serotype distribution has been seen and broad serotype protection is, therefore, desirable.[10] Due to the better response to conjugated vaccines than to T-cell-independent vaccines in CLL patients, the use of the recently available PCV20 may lead to broader serotype protection; moreover, future candidate vaccines incorporating additional serotypes are under development.[29]

Our results also indicate a need for an improved vaccination strategy in CLL patients with low immunoglobulin levels and ongoing/recent leukemia treatment, conditions associated with increased risk of infections and impaired immune response to pneumococcal vaccinations.[6,12-14] Five years after primary immunization, patients with hypogammaglobulinemia had significantly lower GMC for 4/12 serotypes as compared to patients without hypogammaglobulinemia, and after revaccination, GMC were lower for all 12/12 serotypes. According to previous studies, treatment with BTK inhibitors and anti-CD20 antibodies in CLL patients reduces the response to pneumococcal vaccines.[6,8,16] Mauro *et al*. described that none of 44 patients treated with chemoimmunotherapy and only 1/11 patients on continuous BTK inhibitor treatment showed a SR after one dose of PCV13.[6] Also, in a study in CLL patients immunized sequentially with PCV13 and PPSV23, the SR rate was only 2.6% in patients with ongoing therapy.[16] In our study, none of the few patients with ongoing BTK inhibitor and anti-CD20 antibody treatment reached SR or SP after revaccination. Due to the heterogeneic clin-

ical presentation of CLL patients, with various degrees of immunosuppression, future studies may be designed to optimize vaccinations strategies focused on patients with hypogammaglobulinemia and patients with ongoing treatment.

In this study, we used pre-defined SP and SR criteria based on previous studies and according to World Health Organization (WHO) standards.[12,15,16,22,30] The proportions of patients and controls reaching SP according to the cut-off level of 0.35 μg/mL 5 years after primary immunization was 10% in CLL patients and 32% in controls. When evaluating SP according to the more stringent cut-off suggested by Orange *et al.*,[21] the proportions reaching SP decreased to 0% and 6%, respectively. The use of various definitions for SP and SR, and the lack of correlation between vaccine response and susceptibility to infections, restrict the possibility of comparing results between pneumococcal vaccine studies. An additional factor to consider when comparing antibody concentrations is the laboratory method used for measuring serotype-specific IgG levels. The WHO recommends enzyme immunoassays as the standard method, but fluorescent-bead-based multiplex immunoassays have evolved as a time-saving method and are increasingly used by vaccine manufacturers and diagnostic laboratories, including in our study.[31] Although the method is not standardized, the EU Pneumo Multiplex Assay Consortium has shown high agreement between laboratories in Europe.[32] Consensus on how to use surrogate markers for the definition of response and protection in adults and especially in immunocompromised groups of patients is highly warranted. Although this is the largest study on pneumococcal revaccination of CLL patients, a limitation is the low number of patients included in each study arm. However, the comparison between pneumococcal vaccines demonstrates the importance of optimizing vaccination strategies for CLL patients. Moreover, evaluating the immune response after pneumococcal vaccination by measuring serotype-specific circulating antibody concentrations does not necessarily estimate the functionality of antibodies, instead, opsonophagocytic assays need to be performed. It should be noted that we used a 13-valent conjugated pneumococcal vaccine in this study, but our results are also relevant for 15-valent and 20-valent conjugated vaccines.[33]

Antibody titers are commonly measured 4-8 weeks after pneumococcal vaccination. To limit the number of visits, we used an interval of 8 weeks in both groups based on the recommendation that PPSV23 should be administered not earlier than 8 weeks after PCV13. Since a decline in IgG concentrations may occur as early as 4 to 8 weeks after vaccination, the timepoint must be considered when comparing our results to those of previous studies. Moreover, since most patients in this study were treatment-naïve, conclusions cannot be drawn regarding the impact of vaccination in CLL patients receiving specific treatment regimes.

Further studies should evaluate whether repeated vaccinations with a conjugated pneumococcal vaccine should be given as part of primary immunization and the waning of antibody concentrations should be followed in order to identify the optimal timing for revaccination. Additionally, it would be useful to evaluate antibody responses with opsonophagocytic assays and explore dynamics of T, B and NK cell populations after primary vaccination as well as after revaccination with conjugated pneumococcal vaccines in order to determine which strategy of immune stimulation would most effectively activate and retain mucosal, humoral, and cellular immunity in CLL patients. Antibody persistence 5 years after primary immunization with pneumococcal vaccines is impaired in CLL patients compared to that in immunocompetent controls, but revaccination with conjugated pneumococcal vaccines improves immunity in these patients. Our findings support repeated doses of T-cell-dependent pneumococcal vaccines to improve protection in CLL patients further and underline the need for revision of current pneumococcal vaccination recommendations for this high-risk group.

Disclosures

The authors declare that personally they have no conflicts of interest to disclose. Until September 2022, the Finnish Institute for Health and Welfare had a public-private partnership with vaccine manufacturers and has previously received research funding for studies, unrelated to this study, from GlaxoSmithKline Vaccines, Pfizer and Sanofi Pasteur.

Contributions

The study was designed, and the protocol written by MK, BU, EK, TN and SA. P-OA, YH, SL, IN, DR, MS and TT were local Principal Investigators for the study and included patients according to protocol. CV, MM and NE planned and performed the laboratory analyses. MK managed data collection, performed statistical analyses and interpreted data. AM gave statistical advice, performed statistical analyses including the mixed model analysis, and contributed to interpreting the data. MK wrote the manuscript. All authors revised the content and approved the final version.

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Data-sharing statement

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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