haematologica
Journal of the Ferrata Storti Foundation

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

by Magdalena Kättström, Bertil Uggla, Camilla Virta, Merit Melin, Nina Ekström, Anders Magnuson, Per-Ola Andersson, Ylva Hammarlund, Sandra Lockmer, Ingmar Nilsson, Daniel Roth, Magnus Svensson, Tobias Tolf, Eva Kimby, Torbjörn Norén and Simon Athlin

Received: November 8, 2024.
Accepted: February 27, 2025.

Citation: Magdalena Kättström, Bertil Uggla, Camilla Virta, Merit Melin, Nina Ekström, Anders Magnuson, Per-Ola Andersson, Ylva Hammarlund, Sandra Lockmer, Ingmar Nilsson, Daniel Roth, Magnus Svensson, Tobias Tolf, Eva Kimby, Torbjörn Norén and Simon Athlin. Revaccination with pneumococcal conjugate vaccine five years after primary immunization improves immunity in patients with chronic lymphocytic leukemia. Haematologica. 2025 Mar 6. doi: 10.3324/haematol.2024.286942 [Epub ahead of print]

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], Simon Athlin[12]

Affiliations:

1 Section of Hematology, Department of Medicine, Faculty of Medicine and Health, Örebro University, SE 70185, 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

12 School of Medical Sciences, Faculty of Medicine and Health, Örebro University, SE 70185, Örebro, Sweden

Running heads: Pneumococcal revaccination in CLL patients

Corresponding author: Magdalena Kättström, MD,

e-mail: Magdalena.Kattstrom@regionorebrolan.se

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

Competing Interests: The authors declare no competing interests. The Finnish Institute for Health and Welfare has until September 2022 conducted 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.

Author 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 PIs of the study and included patients according to protocol. CV, MM, NE planned and performed the laboratory analysis. MK managed data collection, performed statistical analysis and interpreted data. AM gave statistical advice, performed statistical analysis including mixed model analysis, and contributed to data interpretation. MK wrote the manuscript and all authors have revised the content and approved the final version.

Word count: Abstract = 192, main text = 3363, 3 tables, 3 figures, one supplemental file with Supplemental Methods and Supplemental Tables (5).

Trial registration: www.clinicaltrials.gov (NCT05316831).

Acknowledgements: Gratitude to the Clinical Research Laboratory, Clinical Trials Unit and Örebro biobank in Region Örebro, Sweden and THL in Helsinki, Finland. Gratitude also to the research nurses working with the study and the participating patients from the hematological centers involved.

Funding: The study was supported by grants from the Region Örebro County Research Committee (OLL-840421, OLL-886921, OLL-929805, OLL-916971, Nyckelfonden (OLL-890171, OLL-974753), ALF funding in Region Örebro County and from Uppsala-Örebro Regional Research Council (RFR-930110, RFR-939461), Sweden.

Abstract

Patients with chronic lymphocytic leukemia (CLL) have impaired response to vaccination, which calls for improved vaccination strategies. This study aimed to evaluate antibody persistence five 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 (SP), defined as 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 (RR 2.7 (95% CI 0.5-13.1)), but was lower in patients compared to controls (10% vs 32%; RR 0.3 (0.1-0.7)). Following revaccination with PCV13, serological response (SR), defined as ≥2–fold increase for ≥70% of shared serotypes, was 24% in patients primary immunized with PCV13 vs 12% with PPSV23 (RR 2.0 (0.6-6.9)). A second revaccination with PCV13 significantly improved SR 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 for severe infections and an impaired ability to respond to vaccination (1). Although a majority of patients are asymptomatic at diagnosis and lack 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 early CLL stages and usually progress over time (2-4). In addition to the immune dysfunction, vaccination responses are affected by various treatment modalities, such as treatment including chemotherapy, CD20 monoclonal antibodies, and Bruton´s tyrosine kinase inhibitors (BTKi) (5-8).

Infections caused by *Streptococcus pneumoniae*, such as pneumonia and invasive pneumococcal disease (IPD), 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 by a T-cell independent polysaccharide vaccine containing 23 serotypes (PPSV23) after eight weeks, to broaden the protection (17). Primary immunization with a conjugated pneumococcal vaccine is supported by our previous randomized study in treatment naïve CLL patients, demonstrating a better immune response using PCV13 compared to PPSV23 (13). Vaccination with PPSV23 after PCV13, either as part of primary immunization or as revaccination after five years, has not shown improved immune response in CLL patients (15, 16). To revaccinate with a conjugated vaccine in healthy elderly adults has been proven to be efficient and safe but has not been investigated in CLL patients (18). In addition, repeated doses with conjugated vaccines have shown improved vaccine response in patients with

hematological malignancies after allogenic stem cell transplantation (HSCT) (19), which supports this strategy also in other immunocompromised groups.

Our study aimed to evaluate the antibody persistence five 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 recruited. Participants were included a median of five years after primary immunization and stratified into two revaccination arms based on initial PCV13 or PPSV23 vaccination (Fig. 1). Detailed inclusion and exclusion criteria, as well as additional information regarding study design, study participants and vaccines are listed in 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 (FMIA) was used to quantify serum IgG (µg/mL) 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 response.

Outcomes

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

Statistics

Baseline characteristics were compared using Mann-Whitney test for continuous variables and chi-2 or Fischer 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 (CI). Group, time (five years after primary immunization, eight 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 Fischer exact test were used when mixed model analysis did not converge due to sparse data.

Geometric mean concentration (GMC) of serotype-specific IgG were compared using random intercept linear mixed model and presented as geometric mean ratios (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

Baseline characteristics of CLL patients and controls are presented in Table 1. At inclusion, hypogammaglobulinemia was observed in 18 (25%) CLL patients. Sixty-one 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 last 12 months. No significant differences in baseline characteristics were observed within the CLL cohort (group A versus group B). 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 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 cut-off ≥1.3 µg/mL.

Serotype specific IgG GMCs did not differ significantly between group A and group B (Supplemental Table 1) but when comparing all CLL patients with controls, GMCs were higher for 8/12 serotypes in the control group (Supplemental Table 2).

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 (CI 0.6-6.9); p=0.25, Table 2, Fig. 2). Lower rates of SR were observed in CLL patients compared to controls (18% vs 42 %, RR 0.4 (0.2-0.7), p=0.04, Table 3, Fig. 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 the group A and both control groups, but not in group B (primarily immunized with PPSV23) (Table 2, Fig. 2, Supplemental Table 3).

Serotype specific GMC increased significantly after revaccination for all serotypes in both CLL patients and controls (Supplemental Table 1, 2 and 4). GMCs were significantly higher in 4/12 serotypes in group A compared to group B (Supplemental Table 1). For controls, GMC were significantly higher for 5/12 serotypes in group C (PCV13/PCV13) compared to group D (PPSV23/PCV13) (Supplemental Table 4). GMC of IgG for all 12 serotypes were significantly higher in the controls compared to CLL patients (Supplemental Table 2).

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 significantly increased, from 12% to 30% (p=0.017) (Table 2, Fig 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 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, Fig 2).

Serotype specific IgG GMC increased significantly after the second revaccination in 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 in 4/12 to 1/12 serotypes, Supplemental Table 1).

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 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 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 the 12 months after first revaccination (10 months after second revaccination) and were lower in 11/12 serotypes in group A and in 6/12 in group B. However, GMC were higher for all measured serotypes in both groups compared to before the first revaccination (Supplemental Table 1). GMC were higher in 10/12 serotypes in controls compared to CLL patients and the decline of antibody concentrations was less pronounced (Supplemental Table 2).

Impact of hypogammaglobulinemia and CLL specific treatment on immunity after pneumococcal revaccination.

Hypogammaglobulinemia (HG) was observed in 24% of the CLL patients, equally distributed between group A and B (Table 1). Five years after primary immunization, serotype specific GMC were significantly higher for four of the measured serotypes in non-HG patients, both adjusted and unadjusted to previous vaccination strategy (Supplemental Table 5). After both vaccinations, the difference increased further with significantly higher GMC in the non-HG group for eleven 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 BTKi ibrutinib (n=3) or ongoing or recent treatment (within 12 months) with bendamustin and rituximab (BR) (n=3) reached SR any time point. Only one patient, who discontinued fludarabine, cyclophosphamide and rituximab (FCR) one month prior to the study inclusion, presented with SP with cut-off ≥0.35 µg/mL already at baseline and remained positive during the study period (data not shown).

Pneumococcal carriage and IPD

One of the study participants (in group A) had an asymptomatic pneumococcal carriage at baseline and one (in group B) eight weeks after second revaccination. No events of IPD since the start of the first vaccination study 2013 were reported in the CRF.

Safety of revaccination

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

Discussion

This is, to our knowledge, the first study evaluating serological response after revaccination with conjugated pneumococcal vaccines in CLL patients. Previous studies have demonstrated an increased risk in CLL patients for IPD and an impaired antibody response after pneumococcal vaccination (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 five years after primary immunization with PCV13 or PPSV23 compared to controls, but following revaccination with PCV13, the immunity improved in all participants, also in CLL patients. The strategy to revaccinate CLL patients with two consecutive doses of PCV13, eight weeks apart, was safe and improved the response further.

When Lindström and coworkers previously investigated the serological persistence five 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 five years after primary immunization with PCV13, while those immunized with PPSV23 had even lower protection (5%). Also controls showed a decline of antibody concentration over time, as only one-third had persistent antibody concentrations above SP levels five years after 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 five years after primary immunization with PCV13 (23, 24).

Even though only 18 % of CLL patients reached criteria of 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 primarily immunized with PCV13 as compared to PPSV23 but the differences were non-significant (p=0.25 and p=0.052, respectively), probably due to lack of statistical power. Still, 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 was safe and with robust immune responses, regardless of 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 first revaccination to 30% after 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 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 hematological malignancies previously treated with HSCT (26, 27). This strategy has shown to induce protective antibody levels, with some serotype variability, in 40% of HSCT patients a decade after primary immunization (28) which supports 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 HSCT patients, 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 where administration of PPSV23 after a conjugated pneumococcal vaccine did not improve immunity in CLL patients, neither 8 weeks nor five 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 IPD 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 superior response to conjugated vaccines compared to T-cell independent vaccines in CLL patients, the use of the recently available PCV20 may lead to broader serotype protection and, moreover, future candidate vaccines incorporating additional serotypes are underway (29).

Our results also indicate a need for 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, the HG patients had significantly lower GMC for 4/12 serotypes as compared to non-HG patients, and after revaccination, GMC were lower for all 12/12 serotypes. According to previous studies, treatment with BTKi and anti-CD20 antibodies in CLL patients reduce 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 BTKi treatment showed serological response after one dose of PCV13 (6). Also, in a study in CLL patients immunized sequentially with PCV13 and PPSV23, the serological response rate was only 2.6% in patients with ongoing therapy (16). In our study, none of the few patients with ongoing BTKi and anti-CD20 antibody treatment reached SR or

SP after revaccination. Due to the heterogenic clinical presentation in CLL patients, with various degrees of immunosuppression, future studies may be designed to optimize vaccinations strategies focused on HG patients and patients with ongoing treatment.

In this study, we used pre-defined SP and SR criteria based on previous studies and according to WHO standards (12, 15, 16, 22, 30). Proportion of patients and controls reaching SP according to cut-off level 0.35 µg/mL five 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), proportion 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 to compare 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. WHO recommends the use of enzyme immunoassays as standard method, but the fluorescent-bead-based multiplex immunoassays has evolved as a time-saving method and is used increasingly by vaccine manufacturers and diagnostic laboratories, including in our study (31). The method is not standardized; however, EU Pneumo Multiplex Assay Consortium has shown high agreement between laboratories in Europe (32). Consensus on how to use surrogate markers for definition of response and protection in adults and especially in immunocompromised patient groups is highly warranted.

Although this is the largest study on pneumococcal revaccination of CLL patients, a limitation is the low number of included patients 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 (OPA) need to be

performed. Also, 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 post-vaccination, the timepoint must be considered when comparing our results to previous studies. Moreover, since most patients were treatment naïve in this study, conclusions cannot be drawn regarding the impact of vaccination on CLL patients with specific treatment regimes.

Further studies should evaluate whether repeated vaccinations with a conjugated pneumococcal vaccine should be given as part of primary immunization and follow the waning of antibody concentrations to identify the optimal timing for revaccination. Additionally, evaluating antibody response with OPA and explore dynamics of T, B and NK cell populations after primary as well as after revaccination with conjugated pneumococcal vaccines are warranted to determine which strategy of immune stimulation most effectively would activate and retain mucosal, humoral, and cellular immunity in CLL patients.

CLL patients have an impaired antibody persistence five years after primary immunization with pneumococcal vaccines compared to immunocompetent controls but revaccination with conjugated pneumococcal vaccines improves immunity. 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.

References:

1. Francis ER, Vu J, Perez CO, Sun C. Vaccinations in patients with chronic lymphocytic leukemia. Semin Hematol. 2024;61(2):131-138.

2. Forconi F, Moss P. Perturbation of the normal immune system in patients with CLL. Blood. 2015;126(5):573-581.

3. Parikh SA, Leis JF, Chaffee KG, et al. Hypogammaglobulinemia in newly diagnosed chronic lymphocytic leukemia: Natural history, clinical correlates, and outcomes. Cancer. 2015;121(17):2883-2891.

4. Grywalska E, Zaborek M, Lyczba J, et al. Chronic Lymphocytic Leukemia-Induced Humoral Immunosuppression: A Systematic Review. Cells. 2020;9(11):2398

5. Palma M, Mulder TA, Osterborg A. BTK Inhibitors in Chronic Lymphocytic Leukemia: Biological Activity and Immune Effects. Front Immunol. 2021;12:686768.

6. Mauro FR, Giannarelli D, Galluzzo CM, et al. Response to the conjugate pneumococcal vaccine (PCV13) in patients with chronic lymphocytic leukemia (CLL). Leukemia. 2021;35(3):737-746.

7. Vijenthira A, Gong I, Betschel SD, Cheung M, Hicks LK. Vaccine response following anti-CD20 therapy: a systematic review and meta-analysis of 905 patients. Blood Adv. 2021;5(12):2624-2643.

8. Andrick B, Alwhaibi A, DeRemer DL, et al. Lack of adequate pneumococcal vaccination response in chronic lymphocytic leukaemia patients receiving ibrutinib. Br J Haematol. 2018;182(5):712-714.

9. Andersen MA, Niemann CU, Rostgaard K, et al. Differences and Temporal Changes in Risk of Invasive Pneumococcal Disease in Adults with Hematological Malignancies: Results from a Nationwide 16-Year Cohort Study. Clin Infect Dis. 2021;72(3):463-471.

10. Garcia Garrido HM, Knol MJ, Heijmans J, et al. Invasive pneumococcal disease among adults with hematological and solid organ malignancies: A population-based cohort study. Int J Infect Dis. 2021;106:237-245.

11. Morrison VA. Infectious complications of chronic lymphocytic leukaemia: pathogenesis, spectrum of infection, preventive approaches. Best Pract Res Clin Haematol. 2010;23(1):145-153.

12. Sinisalo M, Vilpo J, Itala M, Vakevainen M, Taurio J, Aittoniemi J. Antibody response to 7-valent conjugated pneumococcal vaccine in patients with chronic lymphocytic leukaemia. Vaccine. 2007;26(1):82-87.

13. Svensson T, Kattstrom M, Hammarlund Y, et al. 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. Vaccine. 2018;36(25):3701-3707.

14. Pasiarski M, Rolinski J, Grywalska E, et al. Antibody and plasmablast response to 13-valent pneumococcal conjugate vaccine in chronic lymphocytic leukemia patients--preliminary report. PLoS One. 2014;9(12):e114966.

15. Lindstrom V, Aittoniemi J, Salmenniemi U, Kayhty H, Huhtala H, Sinisalo M. Antibody response to the 23-valent pneumococcal polysaccharide vaccine after conjugate vaccine in patients with chronic lymphocytic leukemia. Hum Vaccin Immunother. 2019;15(12):2910-2913.

16. Haggenburg S, Garcia Garrido HM, Kant IMJ, et al. Immunogenicity of the 13-Valent Pneumococcal Conjugated Vaccine Followed by the 23-Valent Polysaccharide Vaccine in Chronic Lymphocytic Leukemia. Vaccines (Basel). 2023;11(7):1201

17. Mikulska M, Cesaro S, de Lavallade H, et al. Vaccination of patients with haematological malignancies who did not have transplantations: guidelines from the 2017 European Conference on Infections in Leukaemia (ECIL 7). Lancet Infect Dis. 2019;19(6):e188-e199.

18. Cannon K, Elder C, Young M, et al. A trial to evaluate the safety and immunogenicity of a 20-valent pneumococcal conjugate vaccine in populations of adults >/=65 years of age with different prior pneumococcal vaccination. Vaccine. 2021;39(51):7494-7502.
19. Cordonnier C, Ljungman P, Juergens C et al. Immunogenicity, safety, and tolerability of 13-valent pneumococcal conjugate vaccine followed by 23-valent pneumococcal polysaccharide vaccine in recipients of allogeneic hematopoietic stem cell transplant aged >/=2 years: an open-label study. Clin Infect Dis. 2015;61(3):313-323.
20. Timby N, Hernell O, Vaarala O, Melin M, Lonnerdal B, Domellof M. Infections in infants fed formula supplemented with bovine milk fat globule membranes. J Pediatr Gastroenterol Nutr. 2015;60(3):384-389.
21. Orange JS, Ballow M, Stiehm ER, et al. Use and interpretation of diagnostic vaccination in primary immunodeficiency: a working group report of the Basic and Clinical Immunology Interest Section of the American Academy of Allergy, Asthma & Immunology. J Allergy Clin Immunol. 2012;130(3 Suppl):S1-24.
22. Lindstrom V, Aittoniemi J, Salmenniemi U, et al. Antibody persistence after pneumococcal conjugate vaccination in patients with chronic lymphocytic leukemia. Hum Vaccin Immunother. 2018;14(6):1471-1474.
23. Frenck RW, Jr., Fiquet A, Gurtman A, et al. Immunogenicity and safety of a second administration of 13-valent pneumococcal conjugate vaccine 5 years after initial vaccination in adults 50 years and older. Vaccine. 2016;34(30):3454-3462.
24. Jackson LA, Gurtman A, Rice K, et al. Immunogenicity and safety of a 13-valent pneumococcal conjugate vaccine in adults 70 years of age and older previously vaccinated with 23-valent pneumococcal polysaccharide vaccine. Vaccine. 2013;31(35):3585-3593.
25. Kattstrom M, Uggla B, Tina E, Kimby E, Noren T, Athlin S. Improved plasmablast response after repeated pneumococcal revaccinations following primary immunization with 13-valent pneumococcal conjugate vaccine or 23-valent pneumococcal polysaccharide vaccine in patients with chronic lymphocytic leukemia. Vaccine. 2023;41(19):3128-3136.
26. Wodi AP, Murthy N, McNally VV, Daley MF, Cineas S. Advisory Committee on Immunization Practices Recommended Immunization Schedule for Children and Adolescents Aged 18 Years or Younger - United States, 2024. MMWR Morb Mortal Wkly Rep. 2024;73(1):6-10.
27. Cordonnier C, Einarsdottir S, Cesaro S, et al. Vaccination of haemopoietic stem cell transplant recipients: guidelines of the 2017 European Conference on Infections in Leukaemia (ECIL 7). Lancet Infect Dis. 2019;19(6):e200-e212.
28. Cordonnier C, Labopin M, Robin C, et al. Long-term persistence of the immune response to antipneumococcal vaccines after Allo-SCT: 10-year follow-up of the EBMT-IDWP01 trial. Bone Marrow Transplant. 2015;50(7):978-983.
29. Chichili GR, Smulders R, Santos V, et al. Phase 1/2 study of a novel 24-valent pneumococcal vaccine in healthy adults aged 18 to 64 years and in older adults aged 65 to 85 years. Vaccine. 2022;40(31):4190-4198.
30. Recommendations for the production and control of pneumococcal conjugate vaccines. In: WHO Expert Committee on Biological Standardization. Fifty-fourth report. Geneva, World Health Organization, 2005 (WHO Technical Report Series, No. 927), Annex 2.
31. Andrade DC, Borges IC, Laitinen H, et al. A fluorescent multiplexed bead-based immunoassay (FMIA) for quantitation of IgG against Streptococcus pneumoniae, Haemophilus influenzae and Moraxella catarrhalis protein antigens. J Immunol Methods. 2014;405:130-143.
32. Meek B, Ekstrom N, Kantso B, et al. Multilaboratory Comparison of Pneumococcal Multiplex Immunoassays Used in Immunosurveillance of Streptococcus pneumoniae across Europe. mSphere. 2019;4(6): e00455-e00519
33. Theilacker C, Fletcher MA, Jodar L, Gessner BD. PCV13 Vaccination of Adults against Pneumococcal Disease: What We Have Learned from the Community-Acquired Pneumonia Immunization Trial in Adults (CAPiTA). Microorganisms. 2022;10(1):127

Table 1. Characteristics of CLL patients and controls. Data is presented as median (25th – 75th percentile) or n (%). P was calculated with Mann-Whitney test for continuous variables and chi-2 or Fischer exact test when appropriate for categorical variables. Group A: PCV13 / PCV13 / PPSV13, Group B: PPSV23 / PCV13 / PCV13, Group C PCV13/PCV13, Group D PPSV23/PCV13

	CLL patients					Controls					
	CLL Group A (n=36)		CLL Group B (n=38)			Control Group C (n=9)		Control Group D (n=22)			P CLL vs controls
	n		n		P	n		n		P	
Age, median years (range)	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	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	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, months (range)	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, months (range)	36	83 (66-126)	38	105 (63-154)	0.51		NA		NA		NA
Hypogammaglobulinemia	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 (range)	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 (range)	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 (range)	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 (range)	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
Tretment 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)							

Between groups

	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 ug/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 ug/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

	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 ug/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 ug/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
	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 ug/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 ug/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
	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 ug/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 ug/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						

Table 2. Comparing serological protection (SP) and serological response (SR) between and within group A and B of CLL patients before revaccination (five years after primary immunization), 8 weeks after revaccination 1, 8 weeks after revaccination 2 and 12 months after revaccination 1 with mixed model analysis.

Group A: PCV13 / PCV13 / PPSV13; Group B: PPSV23 / PCV13 / PCV13. RR Relative risk ratio; CI Confidence interval; NA Not applicable; Group A: CLL patients PCV13+PCV13+PPSV13; Group B: CLL patients PPSV23+PCV13+PCV13. [a] SP Serological protection defined as ≥0.35 ug/ml in at least nine (70 %) of the 12 serotypes. [b] SP Serological protection defined as ≥1.3 ug/ml in at least nine (70 %) of the 12 serotypes. [c] SR Serological response defined as 2-fold increase above IgG levels ≥ 0.35 ug/ml in at least nine (70%) of the 12 serotypes compared to baseline. [defghi] Exact McNemar test when 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.

Between groups

	Before revaccination				8 weeks after revaccination 1				12 months 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 ug/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 ug/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

	Change 8 weeks after revaccination vs. before revaccination						Change 12 months after revaccination vs. before revaccination					
	Patients		Controls		Patients vs. Controls		Patients		Controls		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 ug/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 ug/ml [b]	NA	**<0.001** [e]	9.0 (2.4-33.4)	**<0.001**	NA	NA	NA	**0.016** [f]	NA	0.25 [g]	NA	NA
SR 2-fold increase	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	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 ug/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 ug/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						

Table 3. Comparing serological protection (SP) and serological response (SR) between and within CLL patients (group A and B) and controls (group C and D) before revaccination, 8 weeks after revaccination and 12 months after revaccination with mixed model analysis.

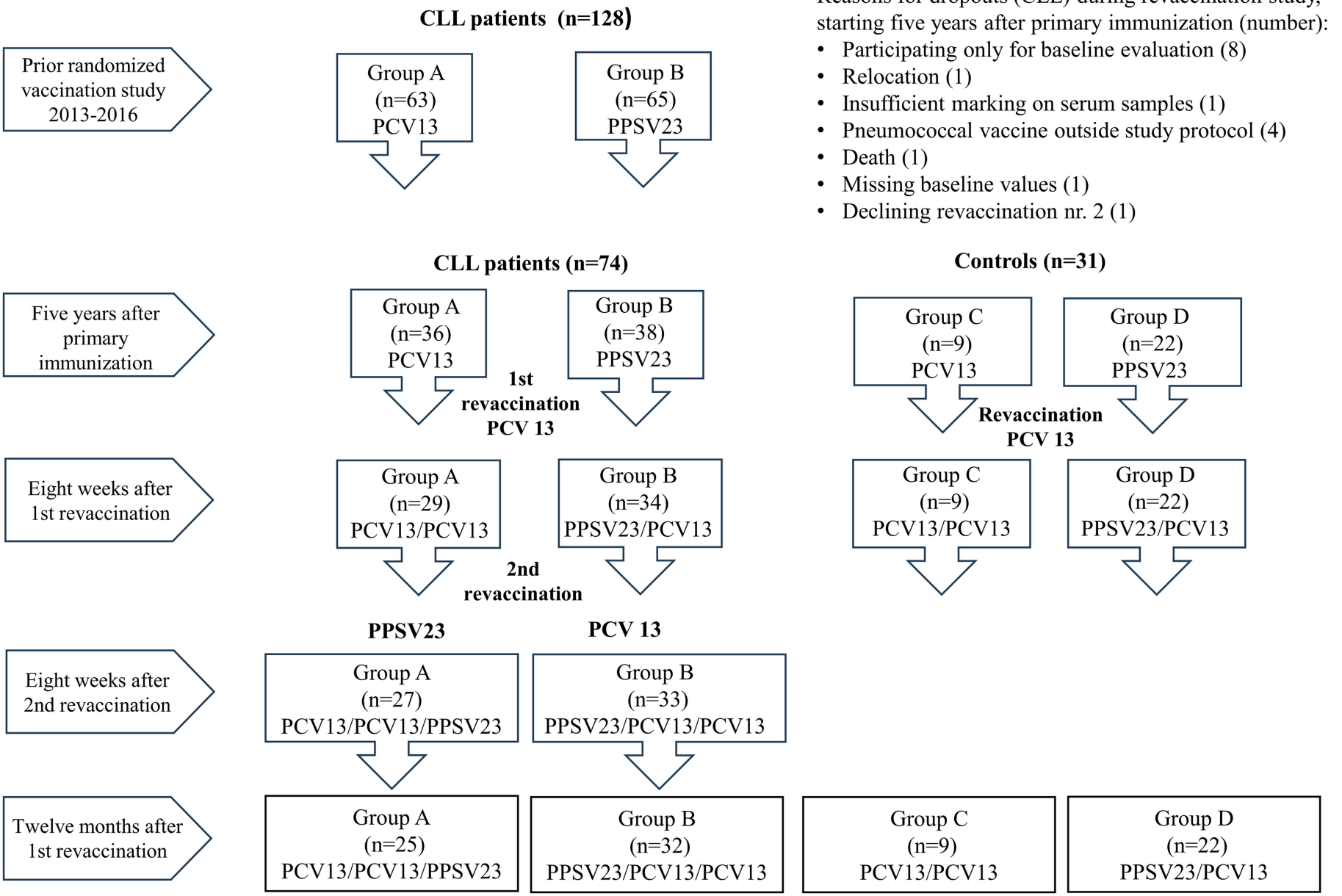
[a] SP Serological protection defined as ≥0.35 ug/ml in at least nine (70 %) of the 12 serotypes. [b] SP Serological protection defined as ≥1.3 ug/ml in at least nine (70 %) of the 12 serotypes. [c] SR Serological response defined as 2-fold increase above IgG levels ≥ 0.35 ug/ml in at least nine (70%) of the 12 serotypes compared to baseline. [d] Adjusted for pre-treatment (PCV13 or PPSV23). In CLL patient group had 36 of 74 (48.6%) and in control group 9 of 31 (29.0%) PCV13 as pre-treatment. [e] Fischer exact test when mixed model analysis did not converge due to sparse data. [fgh] 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; CI Confidence interval; NA Not applicable

Legends to figures:

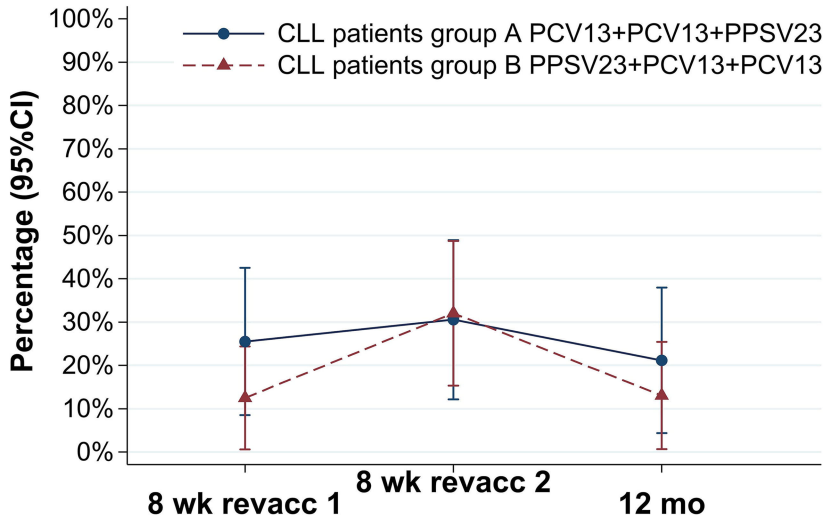
Figure 1. Study design, study population and revaccination strategy among CLL patients and immunocompetent controls.

Figure 2. Serological response and protection in CLL patients. **a)** SR in group A and group B after revaccination 1, after revaccination 2 and 12 months after revaccination 1**.** *SR Serological response defined as 2-fold increase above IgG levels ≥ 0.35 ug/ml in at least nine (70%) of the 12 serotypes compared to baseline.* **b)** SP in group A and group B before revaccination (five years after primary immunization), after revaccination 1, after revaccination 2 and 12 months after revaccination 1**.** *SP Serological protection defined as ≥0.35 ug/ml in at least nine (70 %) of the 12 serotypes.* **c)** SP in group A and group B before revaccination (five years after primary immunization), after revaccination 1, after revaccination 2 and 12 months after revaccination 1**.** *SP Serological protection defined as ≥1.3 ug/ml in at least nine (70 %) of the 12 serotypes.*

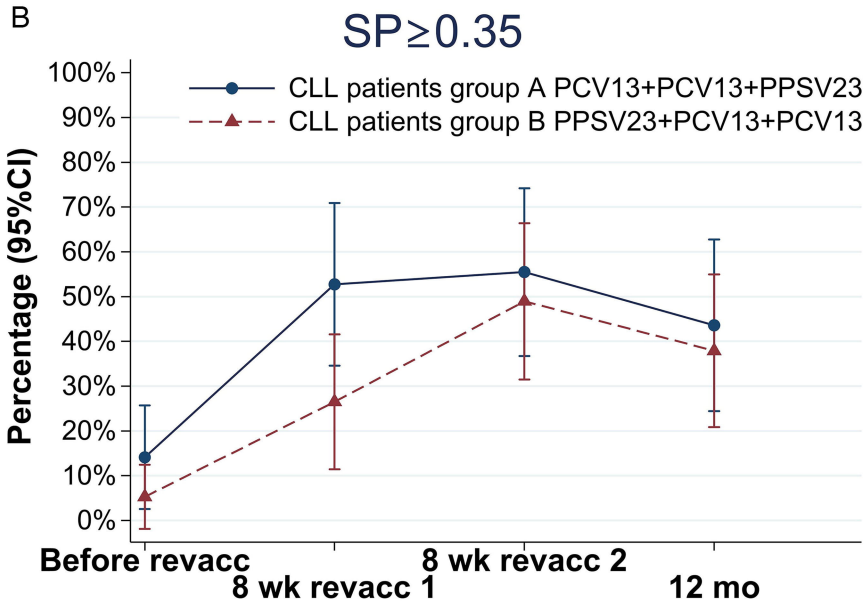
Figure 3. Serological response and protection in CLL patients and controls. **a)** SR in CLL patients and controls after revaccination 1 and 12 months after revaccination. *SR Serological response defined as 2-fold increase above IgG levels ≥ 0.35 ug/ml in at least nine (70%) of the 12 serotypes compared to baseline.* **b)** SP in CLL patients and controls five years after primary immunization, after revaccination 1 and 12 months after revaccination. *SP Serological protection defined as ≥0.35 ug/ml in at least nine (70 %) of the 12 serotypes.* **c)** SP in CLL patients and controls five years after primary immunization, after revaccination 1 and 12 months after revaccination. *SP Serological protection defined as ≥1.3 ug/ml in at least nine (70 %) of the 12 serotypes.*


CLL patients (n=128)
Prior randomized vaccination study 2013-2016
Group A (n=63) PCV13
Group B (n=65) PPSV23
Reasons for dropouts (CLL) during revaccination study, starting five years after primary immunization (number):
• Participating only for baseline evaluation (8)
• Relocation (1)
• Insufficient marking on serum samples (1)
• Pneumococcal vaccine outside study protocol (4)
• Death (1)
• Missing baseline values (1)
• Declining revaccination nr. 2 (1)
CLL patients (n=74)
Controls (n=31)
Five years after primary immunization
Group A (n=36) PCV13
Group B (n=38) PPSV23
1st revaccination PCV 13
Group C (n=9) PCV13
Group D (n=22) PPSV23
Revaccination PCV 13
Eight weeks after 1st revaccination
Group A (n=29) PCV13/PCV13
Group B (n=34) PPSV23/PCV13
Group C (n=9) PCV13/PCV13
Group D (n=22) PPSV23/PCV13
2nd revaccination
PPSV23
PCV 13
Eight weeks after 2nd revaccination
Group A (n=27) PCV13/PCV13/PPSV23
Group B (n=33) PPSV23/PCV13/PCV13
Twelve months after 1st revaccination
Group A (n=25) PCV13/PCV13/PPSV23
Group B (n=32) PPSV23/PCV13/PCV13
Group C (n=9) PCV13/PCV13
Group D (n=22) PPSV23/PCV13

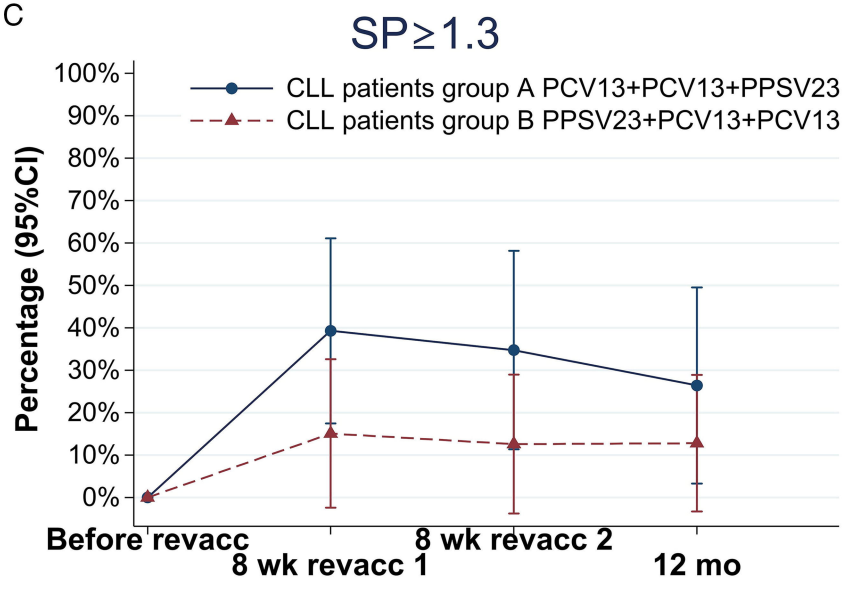
A


SR 2fold
CLL patients group A PCV13+PCV13+PPSV23
CLL patients group B PPSV23+PCV13+PCV13
Percentage (95%CI)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
8 wk revacc 1
8 wk revacc 2
12 mo

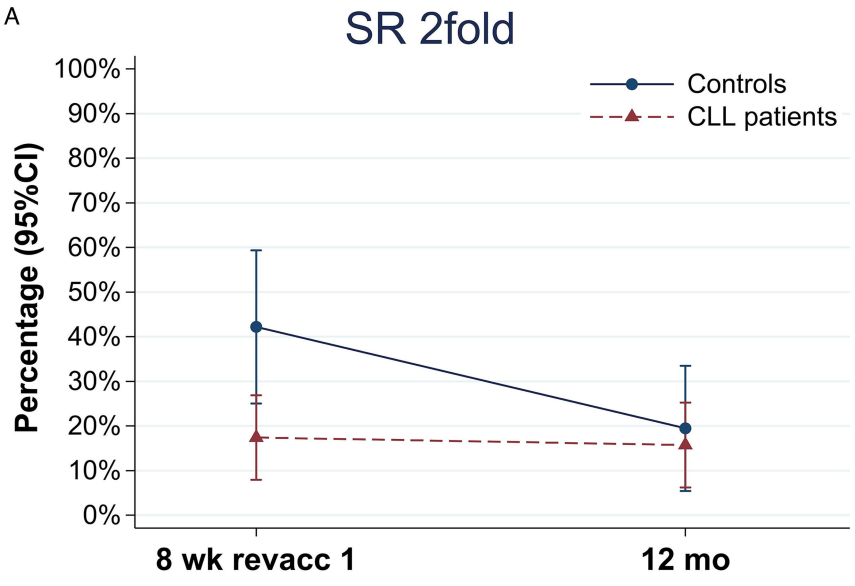
B


SP≥0.35
CLL patients group A PCV13+PCV13+PPSV23
CLL patients group B PPSV23+PCV13+PCV13
Percentage (95%CI)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Before revacc
8 wk revacc 1
8 wk revacc 2
12 mo

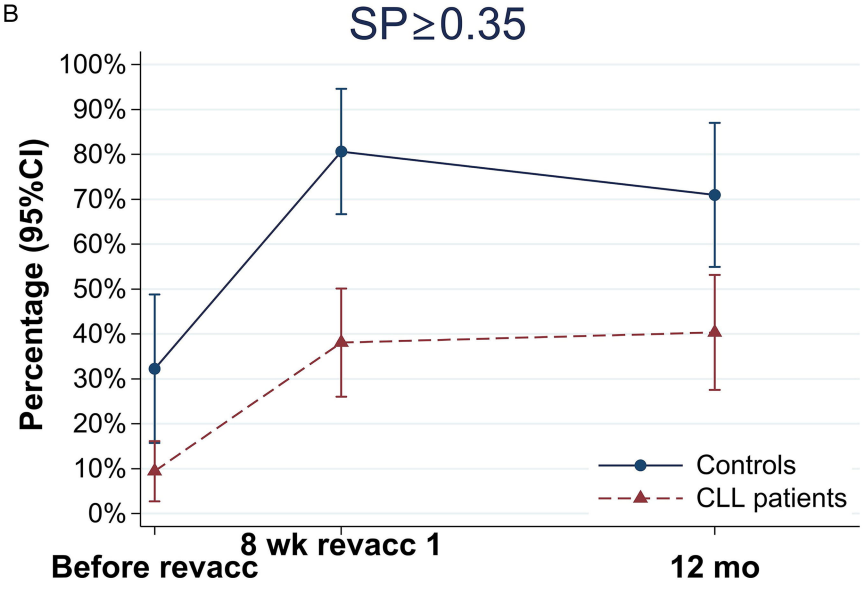
C


SP≥1.3
CLL patients group A PCV13+PCV13+PPSV23
CLL patients group B PPSV23+PCV13+PCV13
Percentage (95%CI)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Before revacc
8 wk revacc 1
8 wk revacc 2
12 mo

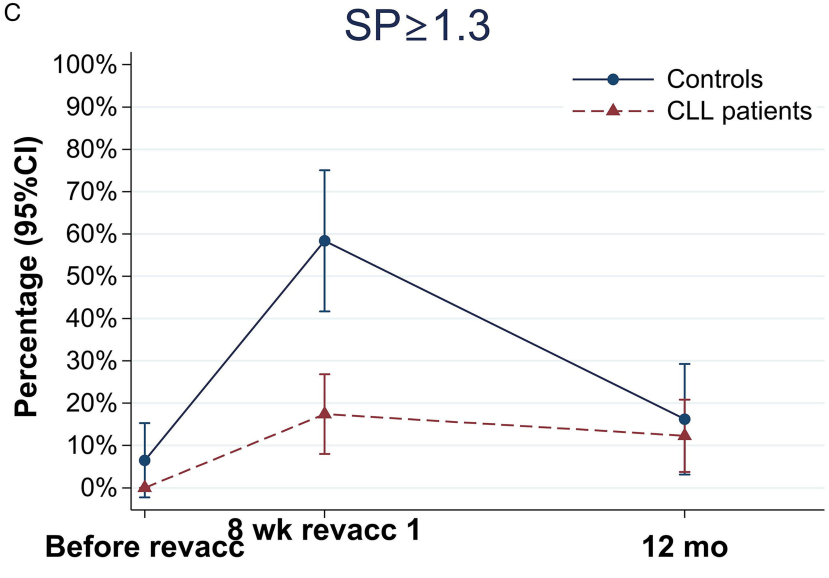
A


SR 2fold
Controls
CLL patients
Percentage (95%CI)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
8 wk revacc 1
12 mo

B


SP≥0.35
Percentage (95%CI)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Controls
CLL patients
Before revacc
8 wk revacc 1
12 mo

C


SP≥1.3
Controls
CLL patients
Percentage (95%CI)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Before revacc
8 wk revacc 1
12 mo

Supplemental Data

Supplemental Methods

1. Inclusion Criteria for Revaccination: CLL patients and Controls

CLL Patients:

CLL patients who were previously enrolled in the Pneumococcal Vaccination Study 0887x1-20003 (EudraCT No: 2009-012642-22) and had received either PCV13 or PPSV23 were eligible for evaluation of long-term immune response. Eligibility for revaccination was determined based on the absence of exclusion criteria. Notably, ongoing or recent CLL-specific treatments were not considered exclusion criteria.

If a patient had received an additional pneumococcal vaccine outside the study protocol after the initial vaccination, the vaccine type and administration date were recorded in the Case Report Form (CRF). If this additional vaccination had been given within the last 12 months, the patient could only participate in the long-term immune response evaluation (Visit 1). However, if more than 12 months had passed since the additional vaccine was administered, the patient became eligible for revaccination.

Control Group:

A control group of immunocompetent individuals that received either PCV13 or PPSV23 approximately 3–5 years earlier was recruited in Region Örebro County through vaccine registries, public notices, and social media. Participants in the control group were eligible for long-term immune response evaluation and revaccination, provided they did not meet any exclusion criteria outlined in the study protocol.

2. Exclusion criteria for revaccination in CLL patients and controls

CLL patients

1. Patients receiving high dose corticosteroids (≥20 mg Prednisolone) or other immunosuppressive drugs that is not part of active CLL treatment (criteria for inclusion after discontinuing high dose corticosteroid treatment, see section 7.3).
2. Patients who have had an allergic reaction to any vaccination in the past.
3. Patients with a positive DAT (Direct Antiglobulin Test) or known present or previous haemolysis, ITP and Guillain-Barre.
4. Patients failing to give informed consent.
5. Patients with ongoing immunoglobulin therapy.
6. Patients with known HIV infection.
7. Patients who have received a pneumococcal vaccine outside the study protocol within the last 12 months.
8. Active febrile infection.
9. Increased bleeding risk due to severe thrombocytopenia or other coagulopathies that would, in the opinion of the investigator, contraindicate intramuscular injection (for treatment with oral anticoagulation therapy, see section 7.3).

Controls

1. Serious chronic disorder including chronic obstructive pulmonary disease (COPD) requiring supplemental oxygen treatment, end-stage renal disease, clinically unstable cardiac disease, or any other disorder that, in the investigator's opinion, excludes the subject from participating in the study.
2. Known or suspected immunodeficiency or other conditions associated with immunosuppression including immunoglobulin class/subclass deficiencies with or without substitution treatment, splenectomy in the medical history, generalized malignancy, human immunodeficiency virus (HIV) infection, haematological malignancies, bone marrow or organ transplant in the medical history.
3. Subjects receiving treatment with high dose corticosteroids (≥20 mg Prednisolone) or other immunosuppressive drugs or planned to receive through study participation.
4. Subjects who have had an allergic reaction to any component of PCV13 in the past.
5. Subjects with known present or previous haemolysis, ITP and Guillain-Barre.
6. Subjects failing to give informed consent.
7. Subjects who have received a pneumococcal vaccine after the primary vaccination approximately 3-5 years ago.
8. Active febrile infection.
9. Increased bleeding risk due to severe thrombocytopenia or other coagulopathies that would, in the opinion of the investigator, contraindicate intramuscular injection (for treatment with oral anticoagulation therapy, see section 7.3).

3 Criteria for temporary delay of vaccine administration

A subject may be included in the study once the condition(s) has/have resolved and no other exclusion criteria are met:

1. Febrile illness (oral temperature ≥100.4°F [38°C]) or other acute illness. Fever and symptoms must have resolved > 48 hours before vaccination.

2. Antibiotic therapy should not have been administered within 72 hours before vaccination

3. Receipt of any inactivated vaccine within 14 days and any live vaccine within 28 days before test vaccination.

4. For patients/controls that are treated with NOAK (Non-vitamin K Oral Anticoagulant) the injection should be given as close as possible to the next dose. After the vaccination, at least two hours should pass before the intake of the next dose. For patients/controls treated with VKA (vitamin K antagonists) with or without one antiplatelet drug, PK should be < 3,0 (measured within 7 days prior to vaccination). For patients/controls treated with VKA in combination with two antiplatelet drugs, PK should be measured the same day and should be ≤1,8. It is recommended to put pressure on the injection site for 10 minutes after vaccination in this patient/control group and to examine the injection site after the patient/control is leaving the clinic.

5. If systemic high dose corticosteroids (≥20 mg Prednisolone) have been administered short term (<14 days) for treatment of an acute illness, subjects should not be enrolled into the study until corticosteroid therapy has been discontinued for at least 28 days before investigational product administration. If systemic high dose corticosteroids have been administered for more than two weeks, a wash-out period of at least 8 weeks is needed before vaccination.

Additional details about the study's design and implementation:

- All tests were performed and analyzed at the local hospital and the results were evaluated according to local reference levels.

- The study was monitored by the Clinical Trials Unit in Region Örebro County.
- The serological assay was performed and validated by the Finnish Institute for Health and Welfare (THL), Helsinki, Finland and accredited by the Finnish Accreditation Service according to accreditation requirement SFS-EN ISO/IEC 17025.
- Pneumococcal vaccines used in the study: PCV13 (Pfizer, Prevenar13®), pneumococcal polysaccharides conjugated to CRM197 carrier protein, and the vaccine includes serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F. PPSV23 (MSD, Pneumovax®) is not conjugated but includes the same serotypes as PCV13, except 6A, and additionally 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20, 22F and 33F. The vaccines were administered by intramuscular injection according to standard recommendations.
- At each study visit, a case report form (CRF) was used, and study participants were monitored with blood samples and nasopharyngeal swabs. Adverse events (AE) and serious adverse events (SAE) were reported until 8 weeks after last revaccination.
- Patients who had received additional pneumococcal vaccinations outside the study protocols were excluded from the analysis.

Supplemental Table 1. Comparison of serotype specific IgG geometric mean concentrations (GMC; µg/ml) and geometric mean ratios (GMR) with 95% confidence intervals (95% CI) between and within group A and B of CLL patients before revaccination, 8 weeks after revaccination 1, 8 weeks after revaccination 2 and 12 months after revaccination 1 with mixed model analysis. GMC Geometric mean concentration; GMR Geometric mean ratio; CI Confidence interval; Group A: CLL patients PCV13+PCV13+PPSV13; Group B: CLL patients PPSV23+PCV13+PCV13.

Between groups

	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	
Serotype	GMC	GMC	GMR (95% CI)	P	GMC	GMC	GMR (95% CI)	P	GMC	GMC	GMR (95% CI)	P	GMC	GMC	GMR (95% CI)	P
1	0.14	0.07	1.9 (0.7-5.0)	0.17	0.60	0.22	3.2 (1.2-8.3)	**0.021**	0.79	0.37	2.3 (0.9-6.1)	0.094	0.41	0.24	2.0 (0.7-5.3)	0.17
3	0.08	0.07	1.1 (0.5-2.2)	0.79	0.20	0.14	1.4 (0.7-2.8)	0.37	0.22	0.25	0.8 (0.4-1.7)	0.66	0.14	0.14	1.0 (0.5-2.0)	0.97
4	0.09	0.03	2.9 (0.9-8.3)	0.050	0.56	0.13	5.3 (1.8-15.7)	**0.003**	0.69	0.22	3.0 (1.0-9.1)	**0.047**	0.41	0.14	2.6 (0.9-7.9)	0.090
5	0.10	0.07	1.4 (0.5-3.5)	0.51	0.40	0.21	2.2 (0.8-5.6)	0.11	0.42	0.22	1.9 (0.7-4.9)	0.20	0.21	0.16	1.5 (0.6-4.0)	0.38
6B	0.11	0.11	1.0 (0.4-2.5)	0.95	0.42	0.21	2.1 (0.8-5.5)	0.13	0.41	0.34	1.1 (0.4-2.9)	0.83	0.22	0.24	1.0 (0.4-2.7)	0.99
7F	0.53	0.27	2.0 (0.8-4.9)	0.14	1.01	0.62	1.9 (0.8-4.9)	0.16	1.35	0.74	1.8 (0.7-4.5)	0.22	1.02	0.54	1.7 (0.7-4.3)	0.27
9V	0.19	0.13	1.4 (0.5-3.7)	0.47	0.64	0.38	1.6 (0.6-4.3)	0.32	0.88	0.51	1.5 (0.6-4.1)	0.39	0.45	0.33	1.4 (0.5-3.6)	0.54
14	0.92	0.62	1.5 (0.5-4.0)	0.43	1.57	0.77	1.8 (0.7-5.0)	0.23	1.78	0.89	1.6 (0.6-4.5)	0.33	1.18	0.76	1.5 (0.6-4.1)	0.41
18C	0.69	0.55	1.2 (0.5-2.9)	0.62	2.38	1.14	2.1 (0.9-5.0)	0.094	2.51	1.47	1.7 (0.7-4.2)	0.22	1.64	1.00	1.6 (0.7-3.9)	0.28
19A	0.60	0.54	1.1 (0.4-2.9)	0.84	1.52	0.85	1.3 (0.5-3.4)	0.59	1.38	1.24	0.8 (0.3-2.0)	0.59	1.02	0.77	0.8 (0.3-2.3)	0.75
19F	0.36	0.20	1.8 (0.7-4.7)	0.23	1.50	0.52	3.1 (1.2-8.4)	**0.023**	1.74	0.86	1.7 (0.6-4.7)	0.27	1.32	0.63	1.6 (0.6-4.3)	0.37
23F	0.36	0.17	2.1 (0.7-6.0)	0.17	1.53	0.40	3.8 (1.3-11.3)	**0.015**	1.43	0.67	2.2 (0.7-6.5)	0.16	0.82	0.40	2.0 (0.7-6.0)	0.20

Within groups

	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	
Serotype	GMR (95% CI)	P	GMR (95% CI)	P	GMR (95% CI)	P	GMR (95% CI)	P	GMR (95% CI)	P	GMR (95% CI)	P
1	4.6 (3.0-7.1)	**<0.001**	2.8 (1.9-4.2)	**<0.001**	1.6 (0.9-2.9)	0.10	5.8 (3.7-9.0)	**<0.001**	4.9 (3.2-7.3)	**<0.001**	1.2 (0.6-2.2)	0.57
3	2.7 (2.0-3.7)	**<0.001**	2.2 (1.6-2.9)	**<0.001**	1.2 (0.8-1.9)	0.31	2.9 (2.1-4.0)	**<0.001**	3.7 (2.7-5.0)	**<0.001**	0.8 (0.5-1.2)	0.30
4	7.2 (4.4-11.8)	**<0.001**	3.9 (2.5-6.2)	**<0.001**	1.8 (0.9-3.6)	0.079	7.7 (4.6-12.9)	**<0.001**	7.3 (4.6-11.7)	**<0.001**	1.0 (0.5-2.1)	0.87
5	4.3 (3.0-6.2)	**<0.001**	2.7 (2.0-3.8)	**<0.001**	1.6 (0.9-2.6)	0.063	4.3 (3.0-6.2)	**<0.001**	3.1 (2.2-4.4)	**<0.001**	1.4 (0.8-2.3)	0.21
6B	4.0 (2.7-5.9)	**<0.001**	1.8 (1.3-2.7)	**<0.001**	2.2 (1.3-3.6)	**0.004**	3.8 (2.6-5.7)	**<0.001**	3.4 (2.3-4.8)	**<0.001**	1.1 (0.7-2.0)	0.62
7F	2.2 (1.6-3.0)	**<0.001**	2.2 (1.7-3.0)	**<0.001**	1.0 (0.6-1.5)	0.95	2.5 (1.8-3.5)	**<0.001**	2.8 (2.1-3.8)	**<0.001**	0.9 (0.6-1.4)	0.95
9V	3.6 (2.5-5.3)	**<0.001**	3.2 (2.3-4.4)	**<0.001**	1.1 (0.7-1.9)	0.57	4.4 (3.0-6.3)	**<0.001**	4.0 (2.9-5.7)	**<0.001**	1.1 (0.6-1.8)	0.77
14	1.7 (1.4-2.2)	**<0.001**	1.4 (1.1-1.7)	**0.002**	1.2 (0.9-1.7)	0.20	1.9 (1.5-2.5)	**<0.001**	1.8 (1.4-2.2)	**<0.001**	1.1 (0.8-1.5)	0.53
18C	3.6 (2.6-5.0)	**<0.001**	2.1 (1.6-2.9)	**<0.001**	1.7 (1.1-2.7)	**0.020**	3.9 (2.8-5.4)	**<0.001**	2.8 (2.0-3.8)	**<0.001**	1.4 (0.9-2.2)	0.15
19A	2.3 (1.7-3.2)	**<0.001**	2.0 (1.5-2.6)	**<0.001**	1.2 (0.8-1.8)	0.43	2.1 (1.6-2.9)	**<0.001**	3.1 (2.3-4.1)	**<0.001**	0.7 (0.4-1.1)	0.089
19F	4.1 (2.7-6.1)	**<0.001**	2.3 (1.6-3.4)	**<0.001**	1.7 (1.0-3.0)	**0.043**	4.1 (2.7-6.2)	**<0.001**	4.2 (2.9-6.1)	**<0.001**	1.0 (0.6-1.7)	0.92
23F	4.3 (2.8-6.6)	**<0.001**	2.4 (1.6-3.5)	**<0.001**	1.8 (1.0-3.2)	**0.037**	4.0 (2.6-6.1)	**<0.001**	3.8 (2.6-5.6)	**<0.001**	1.0 (0.6-1.9)	0.87

	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	
Serotype	GMR (95% CI)	P	GMR (95% CI)	P	GMR (95% CI)	P	GMR (95% CI)	P	GMR (95% CI)	P	GMR (95% CI)	P
1	1.2 (0.8-2.0)	0.32	1.7 (1.1-2.6)	**0.009**	0.7 (0.4-1.3)	0.30	3.3 (2.1-5.3)	**<0.001**	3.2 (2.1-4.9)	**<0.001**	1.0 (0.5-1.9)	0.93
3	1.1 (0.8-1.5)	0.73	1.7 (1.3-2.3)	**<0.001**	0.6 (0.4-0.9)	**0.037**	1.8 (1.3-2.5)	**<0.001**	2.0 (1.5-2.7)	**<0.001**	0.9 (0.6-1.4)	0.64
4	1.1 (0.6-1.8)	0.78	1.9 (1.2-3.0)	**<0.001**	0.6 (0.3-1.2)	0.12	4.3 (2.6-7.3)	**<0.001**	4.8 (3.0-7.7)	**<0.001**	0.9 (0.4-1.8)	0.78
5	1.0 (0.7-1.4)	0.99	1.2 (0.8-1.6)	0.41	0.9 (0.5-1.4)	0.57	2.5 (1.7-3.7)	**<0.001**	2.2 (1.6-3.1)	**<0.001**	1.1 (0.7-1.9)	0.64
6B	1.0 (0.6-1.4)	0.85	1.8 (1.2-2.6)	**0.001**	0.5 (0.3-0.9)	**0.022**	2.4 (1.6-3.6)	**<0.001**	2.3 (1.6-3.4)	**<0.001**	1.0 (0.6-1.8)	0.90
7F	1.1 (0.8-1.6)	0.39	1.3 (0.9-1.7)	0.11	0.9 (0.6-1.4)	0.67	1.8 (1.3-2.5)	**<0.001**	2.1 (1.6-2.8)	**<0.001**	0.8 (0.5-1.3)	0.49
9V	1.2 (0.8-1.7)	0.36	1.3 (0.9-1.8)	0.16	0.9 (0.6-1.6)	0.79	2.5 (1.7-3.7)	**<0.001**	2.6 (1.9-3.7)	**<0.001**	0.9 (0.6-1.6)	0.85
14	1.1 (0.9-1.4)	0.33	1.3 (1.0-1.6)	**0.043**	0.9 (0.6-1.2)	0.52	1.5 (1.2-1.9)	**0.001**	1.5 (1.2-1.8)	**<0.001**	1.0 (0.7-1.4)	0.90
18C	1.1 (0.8-1.5)	0.71	1.3 (0.9-1.8)	0.094	0.8 (0.5-1.3)	0.40	2.6 (1.8-3.6)	**<0.001**	1.9 (1.4-2.7)	**<0.001**	1.3 (0.8-2.1)	0.24
19A	0.9 (0.7-1.2)	0.58	1.6 (1.2-2.1)	**0.002**	0.6 (0.4-0.9)	**0.014**	1.6 (1.2-2.2)	**0.004**	2.1 (1.5-2.8)	**<0.001**	0.8 (0.5-1.2)	0.24
19F	1.0 (0.7-1.5)	0.98	1.8 (1.2-2.6)	**0.002**	0.6 (0.3-0.9)	**0.038**	2.7 (1.8-4.1)	**<0.001**	3.0 (2.1-4.4)	**<0.001**	0.9 (0.5-1.5)	0.65
23F	0.9 (0.6-1.4)	0.73	1.6 (1.1-2.4)	**0.016**	0.6 (0.3-1.0)	0.061	2.5 (1.6-3.8)	**<0.001**	2.6 (1.7-3.8)	**<0.001**	1.0 (0.5-1.7)	0.91

	Change 12 months after revaccination 1 vs. 8 weeks after revaccination 2					
	Group A		Group B		Group A vs. B	
Serotype	GMR (95% CI)	P	GMR (95% CI)	P	GMR (95% CI)	P
1	0.6 (0.4-0.9)	**0.021**	0.7 (0.4-1.0)	0.054	0.9 (0.5-1.6)	0.65
3	0.6 (0.4-0.9)	**0.008**	0.5 (0.4-0.7)	**<0.001**	1.1 (0.7-1.8)	0.54
4	0.6 (0.3-0.9)	**0.035**	0.7 (0.4-1.1)	0.081	0.9 (0.4-1.8)	0.67
5	0.6 (0.4-0.9)	**0.006**	0.7 (0.5-1.0)	0.052	0.8 (0.5-1.4)	0.45
6B	0.6 (0.4-0.9)	**0.028**	0.7 (0.5-1.0)	0.052	0.9 (0.5-1.6)	0.72
7F	0.7 (0.5-0.9)	**0.042**	0.7 (0.6-0.9)	**0.046**	1.0 (0.6-1.5)	0.84
9V	0.6 (0.4-0.9)	**0.006**	0.7 (0.5-0.9)	**0.016**	0.9 (0.5-1.5)	0.64
14	0.8 (0.6-0.9)	**0.042**	0.8 (0.7-1.0)	0.12	0.9 (0.6-1.3)	0.63
18C	0.7 (0.5-0.9)	**0.022**	0.7 (0.5-0.9)	**0.026**	0.9 (0.6-1.5)	0.82
19A	0.7 (0.5-1.0)	0.081	0.7 (0.5-0.9)	**0.007**	1.1 (0.7-1.7)	0.62
19F	0.6 (0.4-0.9)	**0.048**	0.7 (0.5-1.1)	0.089	0.9 (0.5-1.6)	0.73
23F	0.6 (0.4-0.9)	**0.034**	0.7 (0.5-0.9)	**0.046**	0.9 (0.5-1.7)	0.79

Supplemental Table 2. Comparison of IgG antibody concentrations (GMC;µg/ml) between and within CLL patients (group A and B) and controls (group C and D) before revaccination, 8 weeks after revaccination and 12 months after revaccination with mixed model analysis. GMC Geometric mean concentration; GMR Geometric mean ratio; CI Confidence interval. [a] Adjusted for pre-treatment (PCV13 or PPSV23). In CLL patient group had 36 of 74 (48.6%) and in control group 9 of 31 (29.0%) PCV13 as pre-treatment.

Between groups

	Before revaccination				8 weeks after revaccination				12 months after revaccination			
	CLL patients (n=74)	Controls (n=31)	Patients vs. Controls		CLL patients (n=63)	Controls (n=31)	Patients vs. Controls		CLL patients (n=57)	Controls (n=31)	Patients vs. Controls	
Serotype	GMC	GMC	Adjusted GMR [a] (95% CI)	P	GMC	GMC	Adjusted GMR [a] (95% CI)	P	GMC	GMC	Adjusted GMR [a] (95% CI)	P
1	0.10	0.51	0.2 (0.1-0.4)	**<0.001**	0.35	2.81	0.1 (0.04-0.2)	**<0.001**	0.30	1.24	0.2 (0.1-0.5)	**<0.001**
3	0.07	0.14	0.5 (0.3-0.9)	**0.021**	0.17	0.46	0.4 (0.2-0.7)	**0.001**	0.14	0.21	0.6 (0.3-1.2)	0.19
4	0.05	0.23	0.2 (0.1-0.5)	**0.001**	0.26	2.26	0.1 (0.04-0.2)	**<0.001**	0.22	1.00	0.2 (0.1-0.5)	**0.001**
5	0.08	0.27	0.3 (0.1-0.7)	**0.005**	0.28	1.57	0.2 (0.1-0.4)	**<0.001**	0.18	0.67	0.3 (0.1-0.6)	**0.003**
6B	0.11	0.55	0.2 (0.1-0.5)	**<0.001**	0.29	2.95	0.1 (0.03-0.2)	**<0.001**	0.23	1.57	0.2 (0.1-0.4)	**<0.001**
7F	0.37	0.52	0.6 (0.3-1.3)	0.21	0.78	2.66	0.3 (0.1-0.6)	**<0.001**	0.72	1.46	0.4 (0.2-0.9)	**0.038**
9V	0.16	0.27	0.5 (0.2-1.2)	0.12	0.48	1.64	0.3 (0.1-0.6)	**0.002**	0.38	0.78	0.4 (0.2-1.0)	0.062
14	0.75	1.45	0.5 (0.2-1.1)	0.10	1.07	4.80	0.2 (0.1-0.5)	**<0.001**	0.92	3.16	0.3 (0.1-0.8)	**0.011**
18C	0.61	2.75	0.2 (0.1-0.5)	**<0.001**	1.60	9.52	0.2 (0.1-0.3)	**<0.001**	1.24	5.05	0.2 (0.1-0.5)	**<0.001**
19A	0.57	1.58	0.3 (0.1-0.8)	**0.010**	1.11	6.41	0.2 (0.1-0.4)	**<0.001**	0.87	3.21	0.3 (0.1-0.7)	**0.007**
19F	0.27	1.19	0.2 (0.1-0.5)	**<0.001**	0.84	4.59	0.1 (0.06-0.3)	**<0.001**	0.87	2.34	0.3 (0.1-0.7)	**0.004**
23F	0.24	0.52	0.4 (0.2-1.0)	0.063	0.74	2.97	0.2 (0.1-0.5)	**<0.001**	0.55	1.47	0.4 (0.1-0.9)	**0.030**

Within groups

	Change 8 weeks after revaccination vs. baseline						Change 12 months after revaccination vs. baseline					
	CLL patients		Controls		CLL patients vs. Controls		CLL patients		Controls		CLL patients vs. Controls	
Serotype	Adjusted GMR [a] (95% CI)	P	Adjusted GMR [a] (95% CI)	P	Adjusted GMR [a] (95% CI)	P	Adjusted GMR [a] (95% CI)	P	Adjusted GMR [a] (95% CI)	P	Adjusted GMR [a] (95% CI)	P
1	2.8 (1.9-4.0)	**<0.001**	4.7 (3.0-7.3)	**<0.001**	0.6 (0.3-0.9)	**0.047**	3.2 (2.2-4.6)	**<0.001**	2.3 (1.5-3.7)	**<0.001**	1.4 (0.8-2.3)	0.71
3	2.3 (1.7-2.9)	**<0.001**	3.1 (2.2-4.2)	**<0.001**	0.7 (0.5-1.1)	0.10	2.0 (1.5-2.6)	**<0.001**	1.5 (1.1-2.0)	**0.014**	1.4 (0.9-2.0)	0.10
4	3.3 (2.2-5.1)	**<0.001**	7.3 (4.4-12.1)	**<0.001**	0.4 (0.2-0.8)	**0.009**	3.9 (2.5-6.0)	**<0.001**	3.8 (2.3-6.4)	**<0.001**	1.0 (0.6-1.8)	0.97
5	2.7 (1.9-3.9)	**<0.001**	5.1 (3.3-7.7)	**<0.001**	0.5 (0.3-0.9)	**0.012**	2.3 (1.6-3.2)	**<0.001**	2.4 (1.6-3.7)	**<0.001**	0.9 (0.6-1.5)	0.79
6B	1.8 (1.3-2.5)	**<0.001**	4.2 (2.8-6.3)	**<0.001**	0.4 (0.3-0.7)	**<0.001**	2.1 (1.5-3.0)	**<0.001**	2.6 (1.8-4.0)	**<0.001**	0.8 (0.5-1.3)	0.36
7F	2.1 (1.5-2.8)	**<0.001**	4.9 (3.4-7.0)	**<0.001**	0.4 (0.3-0.7)	**<0.001**	2.1 (1.5-2.9)	**<0.001**	2.9 (2.0-4.2)	**<0.001**	0.7 (0.5-1.1)	0.14
9V	2.9 (2.1-4.0)	**<0.001**	5.5 (3.7-8.1)	**<0.001**	0.5 (0.3-0.8)	**0.005**	2.4 (1.7-3.4)	**<0.001**	2.8 (1.9-4.2)	**<0.001**	0.9 (0.5-1.4)	0.54
14	1.3 (0.9-1.6)	0.076	2.9 (2.1-4.0)	**<0.001**	0.4 (0.3-0.6)	**<0.001**	1.4 (1.1-1.9)	**0.008**	2.1 (1.6-2.9)	**<0.001**	0.7 (0.5-0.9)	**0.031**
18C	2.2 (1.6-3.0)	**<0.001**	3.0 (2.1-4.4)	**<0.001**	0.7 (0.5-1.1)	0.16	2.0 (1.5-2.8)	**<0.001**	1.7 (1.2-2.5)	**0.004**	1.2 (0.7-1.8)	0.48
19A	2.0 (1.5-2.6)	**<0.001**	3.9 (2.8-5.4)	**<0.001**	0.5 (0.3-0.8)	**<0.001**	2.0 (1.5-2.6)	**<0.001**	2.1 (1.5-2.9)	**<0.001**	0.9 (0.6-1.4)	0.73
19F	2.4 (1.8-3.4)	**<0.001**	3.4 (2.3-4.9)	**<0.001**	0.7 (0.5-1.1)	0.16	2.9 (2.1-4.1)	**<0.001**	2.0 (1.3-2.9)	**<0.001**	1.5 (0.9-2.3)	0.10
23F	2.1 (1.5-3.0)	**<0.001**	4.5 (2.9-6.8)	**<0.001**	0.5 (0.3-0.8)	**0.003**	2.2 (1.6-3.2)	**<0.001**	2.6 (1.7-4.0)	**<0.001**	0.8 (0.5-1.4)	0.55

	Change 12 months vs. 8 weeks after revaccination					
	CLL patients		Controls		CLL patients vs. Controls	
Serotype	Adjusted GMR [a] (95% CI)	P	Adjusted GMR [a] (95% CI)	P	Adjusted GMR [a] (95% CI)	P
1	1.1 (0.8-1.7)	0.51	0.5 (0.3-0.8)	**0.002**	2.3 (1.3-3.9)	**0.002**
3	0.9 (0.7-1.2)	0.36	0.5 (0.3-0.7)	**<0.001**	1.8 (1.3-2.7)	**0.001**
4	1.2 (0.7-1.8)	0.49	0.5 (0.3-0.9)	**0.013**	2.2 (1.2-4.0)	**0.009**
5	0.8 (0.6-1.2)	0.31	0.5 (0.3-0.7)	**<0.001**	1.7 (1.1-2.8)	**0.027**
6B	1.2 (0.8-1.7)	0.36	0.6 (0.4-0.9)	**0.030**	1.9 (1.1-3.0)	**0.012**
7F	1.0 (0.7-1.4)	0.98	0.6 (0.4-0.9)	**0.006**	1.7 (1.1-2.6)	**0.019**
9V	0.8 (0.6-1.2)	0.33	0.5 (0.3-0.8)	**<0.001**	1.6 (1.0-2.6)	**0.032**
14	1.1 (0.9-1.5)	0.36	0.7 (0.5-0.9)	**0.042**	1.6 (1.1-2.2)	**0.017**
18C	0.9 (0.7-1.3)	0.62	0.6 (0.4-0.8)	**0.004**	1.6 (1.0-2.5)	**0.035**
19A	1.0 (0.7-1.3)	0.94	0.5 (0.4-0.8)	**<0.001**	1.8 (1.2-2.6)	**0.002**
19F	1.2 (0.9-1.7)	0.26	0.6 (0.4-0.9)	**0.009**	2.0 (1.3-3.2)	**0.002**
23F	1.1 (0.7-1.5)	0.75	0.6 (0.4-0.9)	**0.015**	1.8 (1.1-3.0)	**0.022**

Supplemental Table 3. Comparison of serological protection (SP) and serological response (SR) between and within group C and D of controls before revaccination, 8 weeks after revaccination and 12 months after revaccination with mixed model analysis. RR Relative risk ratio; CI Confidence interval; NA Not applicable; Group C: Controls PCV13 / PCV13; Group D: Controls PPSV23 / PCV13. [a] SP Serological protection defined as ≥0.35 ug/ml in at least nine (70 %) of the 12 serotypes. [b] SP Serological protection defined as ≥1.3 ug/ml in at least nine (70 %) of the 12 serotypes. [c] SR Serological response defined as 2-fold increase above IgG levels ≥ 0.35 ug/ml in at least nine (70%) of the 12 serotypes compared to baseline. [d] Fisher exact test when mixed model analysis did not converge due to sparse data [efg] Exact McNemar test when mixed model analysis did not converge due to sparse data e0 of 22 vs. 10 of 22, f 2 of 9 vs. 3 of 9, g 0 of 22 vs. 2 of 22

Between groups

	Before revaccination				8 weeks after revaccination				12 months after revaccination			
	Group C (n=9)	Group D (n=22)	Group C vs. D		Group C (n=9)	Group D (n=22)	Group C vs. D		Group C (n=9)	Group D (n=22)	Group C vs. D	
	n (%)	n (%)	RR (95% CI)	P	n (%)	n (%)	RR (95% CI)	P	n (%)	n (%)	RR (95% CI)	P
SP ≥ 0.35 ug/ml [a]	4 (44.4)	6 (27.3)	1.6 (0.6-4.5)	0.35	9 (100.0)	16 (72.7)	1.4 (1.1-1.8)	**0.016**	8 (88.9)	14 (63.6)	1.4 (0.9-2.1)	0.10
SP ≥ 1.3 ug/ml [b]	2 (22.2)	0 (0.0)	NA	0.077 [d]	8 (88.9)	10 (45.4)	2.0 (1.2-3.3)	**0.012**	3 (33.3)	2 (9.1)	3.7 (0.7-18.9)	0.12
SR 2 fold increase [c]	NA	NA	NA	NA	5 (55.6)	8 (36.4)	1.5 (0.7-3.5)	0.31	4 (44.4)	2 (9.1)	4.9 (1.0-22.7)	**0.043**

Within groups

	Change 8 weeks after revaccination vs. before revaccination						Change 12 months after revaccination vs. before revaccination					
	Group C		Group D		Group C vs. D		Group C		Group D		Group C vs. D	
	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 ug/ml [a]	2.2 (1.1-4.7)	**0.032**	2.7 (1.4-5.1)	**0.003**	0.8 (0.3-2.3)	0.73	2.0 (0.9-4.0)	0.054	2.3 (1.2-4.6)	**0.016**	0.9 (0.3-2.3)	0.76
SP ≥ 1.3 ug/ml [b]	4.0 (1.2-13.5)	**0.026**	NA	**0.002**[e]	NA	NA	NA	0.99[f]	NA	0.50[g]	NA	NA
SR 2 fold increase [c]	NA		NA		NA	NA	NA	NA	NA	NA	NA	NA

	Change 12 months vs. 8 weeks after revaccination					
	Group C		Group D		Group C vs. D	
	RR (95% CI)	P	RR (95% CI)	P	RR (95% CI)	P
SP ≥ 0.35 ug/ml [a]	0.9 (0.7-1.1)	0.33	0.9 (0.7-1.1)	0.16	1.0 (0.7-1.4)	0.92
SP ≥ 1.3 ug/ml [b]	0.4 (0.1-0.9)	**0.034**	0.2 (0.1-0.7)	**0.012**	1.9 (0.4-8.9)	0.43
SR 2 fold increase [c]	0.8 (0.5-1.2)	0.33	0.2 (0.1-0.8)	**0.026**	3.2 (0.9-11.7)	0.079

Supplemental Table 4. Comparison of serotype specific IgG antibody concentrations (GMC; µg/ml) between and within group C and D of controls before revaccination, 8 weeks after revaccination and 12 months after revaccination with mixed model analysis. GMC Geometric mean concentration; Geometric mean ratio; CI Confidence interval; Group C: Controls PCV13 / PCV13; Group D: Controls PPSV23 / PCV13

Between groups

	Before revaccination				8 weeks after revaccination				12 months after revaccination			
	Group C (n=9)	Group D (n=22)	Group C vs. D		Group C (n=9)	Group D (n=22)	Group C vs. D		Group C (n=9)	Group D (n=22)	Group C vs. D	
Serotype	GMC	GMC	GMR (95% CI)	P	GMC	GMC	GMR (95% CI)	P	GMC	GMC	GMR (95% CI)	P
1	0.47	0.53	0.9 (0.2-3.0)	0.84	4.01	2.43	1.6 (0.5-5.7)	0.43	1.35	1.20	1.1 (0.3-3.9)	0.85
3	0.31	0.10	3.0 (1.1-8.3)	**0.034**	0.92	0.34	2.7 (0.9-7.3)	0.058	0.40	0.16	2.6 (0.9-7.1)	0.068
4	0.12	0.30	0.4 (0.1-2.0)	0.27	4.28	1.74	2.5 (0.5-12.3)	0.27	1.40	0.87	1.6 (0.3-8.0)	0.56
5	0.36	0.24	1.5 (0.3-7.6)	0.60	3.02	1.20	2.5 (0.5-12.5)	0.26	0.92	0.59	1.6 (0.3-7.7)	0.59
6B	1.52	0.36	4.1 (0.9-18.7)	0.065	16.14	1.47	11.0 (2.4-49.6)	**0.002**	6.59	0.87	7.5 (1.7-34.1)	**0.009**
7F	1.04	0.40	2.6 (0.9-7.1)	0.060	7.53	1.73	4.3 (1.6-11.8)	**0.004**	2.65	1.14	2.3 (0.8-6.3)	0.10
9V	0.98	0.16	6.2 (1.7-23.3)	**0.006**	11.15	0.75	14.9 (4.0-55.7)	**<0.001**	4.08	0.40	10.2 (2.7-38.2)	**<0.001**
14	1.99	1.28	1.6 (0.4-6.0)	0.52	12.89	3.21	4.0 (1.0-15.6)	**0.045**	5.11	2.60	2.0 (0.5-7.6)	0.33
18C	2.78	2.74	1.0 (0.3-3.1)	0.98	11.41	8.83	1.3 (0.4-3.9)	0.65	4.89	5.12	0.9 (0.3-2.9)	0.93
19A	2.11	1.40	1.5 (0.5-4.4)	0.46	8.46	5.72	1.5 (0.5-4.4)	0.48	4.16	2.89	1.4 (0.5-4.2)	0.51
19F	1.75	1.02	1.7 (0.5-5.6)	0.37	8.30	3.60	2.3 (0.7-7.5)	0.17	3.53	1.98	1.8 (0.5-5.8)	0.34
23F	0.51	0.52	1.0 (0.2-3.9)	0.96	8.04	1.97	4.1 (1.0-16.5)	**0.049**	2.70	1.15	2.4 (0.6-9.5)	0.23

Within groups

	Change 8 weeks after revaccination vs. before revaccination						Change 12 months after revaccination vs. before revaccination					
	Group C		Group D		Group C vs. D		Group C		Group D		Group C vs. D	
Serotype	GMR (95% CI)	P	GMR (95% CI)	P	GMR (95% CI)	P	GMR (95% CI)	P	GMR (95% CI)	P	GMR (95% CI)	P
1	8.6 (4.3-17.0)	**<0.001**	4.6 (2.9-7.1)	**<0.001**	1.9 (0.8-4.2)	0.13	2.9 (1.4-5.7)	**0.002**	2.2 (1.4-3.5)	**<0.001**	1.3 (0.6-2.9)	0.55
3	2.9 (1.7-5.0)	**<0.001**	3.3 (2.3-4.7)	**<0.001**	0.9 (0.5-1.7)	0.71	1.3 (0.7-2.2)	0.36	1.5 (1.1-2.1)	**0.020**	0.8 (0.4-1.6)	0.63
4	35.0 (17.5-69.8)	**<0.001**	5.7 (3.6-8.8)	**<0.001**	6.2 (2.7-14.0)	**<0.001**	11.4 (5.7-22.8)	**<0.001**	2.8 (1.8-4.4)	**<0.001**	4.0 (1.8-9.1)	**<0.001**
5	8.3 (3.7-18.3)	**<0.001**	5.0 (3.0-8.4)	**<0.001**	1.6 (0.6-4.2)	0.30	2.5 (1.1-5.6)	**0.023**	2.5 (1.5-4.1)	**<0.001**	1.0 (0.4-2.6)	0.98
6B	10.6 (5.5-20.5)	**<0.001**	4.0 (2.6-6.1)	**<0.001**	2.6 (1.2-5.8)	**0.014**	4.3 (2.2-8.4)	**<0.001**	2.4 (1.6-3.6)	**<0.001**	1.8 (0.8-4.0)	0.13
7F	7.2 (3.6-14.7)	**<0.001**	4.4 (2.8-6.9)	**<0.001**	1.7 (0.7-3.9)	0.24	2.6 (1.2-5.2)	**0.010**	2.9 (1.8-4.5)	**<0.001**	0.9 (0.4-2.1)	0.78
9V	11.3 (5.9-21.8)	**<0.001**	4.7 (3.1-7.2)	**<0.001**	2.4 (1.1-5.2)	**0.029**	4.1 (2.1-8.0)	**<0.001**	2.5 (1.7-3.9)	**<0.001**	1.6 (0.7-3.6)	0.22
14	6.5 (3.4-12.3)	**<0.001**	2.5 (1.7-3.8)	**<0.001**	2.6 (1.2-5.5)	**0.014**	2.6 (1.3-4.9)	**0.004**	2.0 (1.3-3.1)	**<0.001**	1.3 (0.6-2.7)	0.54
18C	4.1 (2.1-8.0)	**<0.001**	3.2 (2.1-4.9)	**<0.001**	1.3 (0.6-2.8)	0.55	1.8 (0.9-3.4)	0.096	1.9 (1.2-2.9)	**0.004**	0.9 (0.4-2.1)	0.88
19A	4.0 (2.3-6.9)	**<0.001**	4.1 (2.9-5.8)	**<0.001**	1.0 (0.5-1.9)	0.96	2.0 (1.1-3.4)	**0.014**	2.1 (1.4-2.9)	**<0.001**	1.0 (0.5-1.8)	0.90
19F	4.7 (2.8-7.9)	**<0.001**	3.5 (2.6-4.9)	**<0.001**	1.3 (0.7-2.4)	0.34	2.0 (1.2-3.3)	**0.007**	1.9 (1.4-2.7)	**<0.001**	1.0 (0.6-1.9)	0.91
23F	15.8 (8.6-28.9)	**<0.001**	3.8 (2.5-5.5)	**<0.001**	4.2 (2.0-8.6)	**<0.001**	5.3 (2.9-9.7)	**<0.001**	2.2 (1.5-3.2)	**<0.001**	2.4 (1.2-5.0)	**0.015**

	Change 12 months after revaccination vs. 8 weeks after revaccination					
	Group C		Group D		Group C vs. D	
Serotype	GMR (95% CI)	P	GMR (95% CI)	P	GMR (95% CI)	P
1	0.3 (0.2-0.7)	**0.002**	0.5 (0.3-0.8)	**0.002**	0.7 (0.3-1.5)	0.36
3	0.4 (0.2-0.8)	**0.003**	0.5 (0.3-0.6)	**<0.001**	1.0 (0.5-1.8)	0.91
4	0.3 (0.2-0.7)	**0.002**	0.5 (0.3-0.8)	**0.002**	0.6 (0.3-1.5)	0.31
5	0.3 (0.1-0.7)	**0.003**	0.5 (0.3-0.8)	**0.006**	0.6 (0.2-1.6)	0.31
6B	0.4 (0.2-0.8)	**0.007**	0.6 (0.4-0.9)	**0.014**	0.7 (0.3-1.5)	0.35
7F	0.4 (0.2-0.7)	**0.004**	0.7 (0.4-1.0)	0.073	0.5 (0.2-1.2)	0.14
9V	0.4 (0.2-0.7)	**0.003**	0.5 (0.3-0.8)	**0.003**	0.7 (0.3-1.5)	0.34
14	0.4 (0.2-0.8)	**0.004**	0.8 (0.5-1.2)	0.31	0.5 (0.2-1.0)	0.065
18C	0.4 (0.2-0.8)	**0.012**	0.6 (0.4-0.9)	**0.012**	0.7 (0.3-1.6)	0.45
19A	0.5 (0.3-0.8)	**0.010**	0.5 (0.4-0.7)	**<0.001**	1.0 (0.5-1.9)	0.94
19F	0.4 (0.3-0.7)	**<0.001**	0.5 (0.4-0.8)	**<0.001**	0.8 (0.4-1.4)	0.41
23F	0.3 (0.2-0.6)	**<0.001**	0.6 (0.4-0.9)	**0.006**	0.6 (0.3-1.2)	0.13

Supplemental Table 5. Comparison of serotype specific IgG antibody concentrations (GMC; µg/ml) between patients with and without hypogammaglobulinemia (HG) among KLL patients (group A and B) with mixed model analysis.

[a] Adjusted for group (A or B); HG hypogammaglobulinemia; GMC Geometric mean concentration; GMR Geometric mean ratio; CI Confidence interval.

	Before revaccination				8 weeks after revaccination 1				8 weeks after revaccination 2				12 months after revaccination 1			
	HG (n=18)	no HG (n=55)	HG vs. no HG		HG (n=16)	no HG (n=47)	HG vs. no HG		HG (n=15)	no HG (n=45)	HG vs. no HG		HG (n=13)	no HG (n=44)	HG vs. no HG	
Serotype	GMC	GMC	GMR[a] (95% CI)	P[a]	GMC	GMC	GMR[a] (95% CI)	P[a]	GMC	GMC	GMR[a] (95% CI)	P[a]	GMC	GMC	GMR[a] (95% CI)	P[a]
1	0.05	0.13	0.4 (0.1-1.0)	0.051	0.08	0.58	0.1 (0.04-0.3)	**<0.001**	0.08	0.96	0.1 (0.04-0.3)	**<0.001**	0.06	0.48	0.1 (0.04-0.4)	**<0.001**
3	0.06	0.08	0.7 (0.3-1.6)	0.45	0.10	0.20	0.4 (0.2-0.9)	**0.037**	0.08	0.34	0.3 (0.1-0.6)	**<0.001**	0.08	0.17	0.4 (0.2-0.9)	**0.024**
4	0.02	0.08	0.2 (0.1-0.5)	**0.002**	0.05	0.46	0.1 (0.03-0.3)	**<0.001**	0.05	0.69	0.1 (0.02-0.2)	**<0.001**	0.04	0.37	0.1 (0.03-0.3)	**<0.001**
5	0.08	0.09	0.9 (0.3-2.5)	0.80	0.15	0.35	0.3 (0.1-0.9)	**0.030**	0.12	0.39	0.2 (0.1-0.7)	**0.009**	0.07	0.24	0.3 (0.1-0.9)	**0.036**
6B	0.08	0.13	0.6 (0.2-1.7)	0.32	0.08	0.46	0.2 (0.1-0.5)	**0.002**	0.08	0.62	0.1 (0.05-0.4)	**<0.001**	0.05	0.38	0.2 (0.1-0.5)	**0.002**
7F	0.11	0.54	0.2 (0.1-0.5)	**0.001**	0.22	1.20	0.1 (0.05-0.4)	**<0.001**	0.29	1.45	0.1 (0.05-0.3)	**<0.001**	0.23	1.01	0.1 (0.05-0.4)	**<0.001**
9V	0.06	0.22	0.3 (0.1-0.7)	**0.012**	0.11	0.81	0.1 (0.05-0.4)	**<0.001**	0.09	1.24	0.1 (0.03-0.3)	**<0.001**	0.07	0.61	0.1 (0.04-0.4)	**<0.001**
14	0.51	0.86	0.6 (0.2-1.8)	0.34	0.59	1.31	0.4 (0.1-1.1)	0.083	0.51	1.63	0.3 (0.1-0.9)	**0.044**	0.37	1.21	0.3 (0.1-1.0)	0.053
18C	0.31	0.77	0.4 (0.1-1.0)	0.058	0.55	2.30	0.2 (0.1-0.6)	**0.002**	0.58	2.76	0.2 (0.1-0.5)	**0.001**	0.38	1.76	0.2 (0.1-0.6)	**0.002**
19A	0.29	0.68	0.4 (0.1-1.2)	0.11	0.44	1.53	0.3 (0.1-0.8)	**0.021**	0.36	2.00	0.2 (0.1-0.6)	**0.005**	0.30	1.19	0.2 (0.1-0.8)	**0.013**
19F	0.11	0.38	0.3 (0.1-0.8)	**0.013**	0.22	1.34	0.2 (0.1-0.5)	**<0.001**	0.23	2.03	0.1 (0.04-0.3)	**<0.001**	0.20	1.34	0.1 (0.04-0.4)	**<0.001**
23F	0.13	0.29	0.4 (0.1-1.4)	0.15	0.16	1.27	0.1 (0.03-0.4)	**<0.001**	0.16	1.71	0.1 (0.03-0.3)	**<0.001**	0.11	0.88	0.1 (0.04-0.5)	**0.002**