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# Immunogenicity of a 5-dose pneumococcal vaccination schedule following allogeneic hematopoietic stem cell transplantation

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## Abstract

The optimal schedule of pneumococcal vaccination after allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains controversial. The objective of this study was to investigate the immunogenicity of a 5-dose pneumococcal vaccination schedule in adult allo-HSCT recipients with and without immunosuppressive therapy. In this prospective cohort study, allo-HSCT recipients received four doses of the 13-valent pneumococcal conjugate vaccine (PCV13) and one dose of the 23-valent pneumococcal polysaccharide vaccine (PPSV23) starting 4–6 months after allo-HSCT. PCV13 was administered at T0, T1, T2, and T8 (T = months from enrollment) and PPSV23 at T10. Serum was collected at T0, T4, T8, T10, and T12, and IgG levels were measured for all 24 vaccine serotypes by immunoassay. The primary outcome was overall seroprotection at T12 defined as an IgG concentration ≥1.3 μg/ml for 17/24 vaccine serotypes in allo-HCST recipients with and without immunosuppressive therapy at baseline. Secondary outcomes were serotype-specific seroprotection and dynamics of IgG levels. We included 89 allo-HSCT recipients in the final analysis. Overall seroprotection was 47% (15/32) for patients without immunosuppressive therapy at baseline versus 24% (11/46) for patients with immunosuppressive therapy ($p = .03$). Seroprotection was higher for PCV13 serotypes (78% and 54% respectively; $p = .03$) and lower for PPSV23-unique serotypes (28% and 13% respectively; $p = .1$). IgG concentrations increased significantly over time for all 24 serotypes. Concluding, although immunogenicity of PCV13 serotypes was reasonable, the poor response to PPSV23 serotypes resulted in an insufficient overall response to pneumococcal vaccination for allo-HSCT recipients. Research into vaccination strategies with higher-valent T-cell-dependent pneumococcal vaccines is needed.

# 1 | BACKGROUND

Following allogeneic hematopoietic stem cell transplantation (allo-HSCT), patients are at high risk of invasive pneumococcal disease (IPD), with a reported incidence of 812/100 000 among allo-HSCT recipients compared to 10/100 000 in the general population.[1] Therefore, pneumococcal vaccination is recommended for all allo-HSCT recipients. However, the effectiveness and optimal timing and schedule of pneumococcal vaccination following allo-HSCT remain unclear.[2] European and United States (US) guidelines recommend starting pneumococcal vaccination with a 4-dose vaccination scheduled consisting of 3 priming doses of the 13-valent pneumococcal conjugate vaccine (PCV13) from 3–6 months after allo-HSCT, followed by either a 23-valent pneumococcal polysaccharide vaccine (PPSV23) 6 months later, or a fourth PCV13 in case of chronic graft-versus-host disease (GvHD).[3,4] In a prior publication, we proposed the concept of a 5-dose vaccination schedule for allo-HSCT recipients, consisting of the same 3 PCV13 priming doses 3–6 months after allo-HSCT, followed by a PCV13 booster dose 6 months later and a dose of PPSV23 8 months later, regardless of the presence of GVHD.[2] A similar vaccination schedule was previously evaluated in a cohort of 251 allo-HSCT recipients and showed good serologic responses against PCV13 serotypes (83%–99% after dose 4).[5] Serotypes exclusive to PPSV23 were not reported. However, surveillance data show that IPD caused by these serotypes has become more common, causing 44% of IPD cases, compared to PCV13 serotypes causing only 30%, and nonvaccine serotypes 26% of cases.[6–8]

Therefore, the main objective of this study was to investigate the immune response to all 24 serotypes included in the PCV13 and PPSV23 vaccines, following a 5-dose vaccination schedule in adult allo-HSCT recipients with and without immunosuppressive therapy at baseline. In addition, we investigated clinical and laboratory parameters for their value to predict response to vaccination.

# 2 | METHODS

We performed a multicenter prospective cohort study at the Amsterdam University Medical Centers, location AMC and location VuMC, between August 2018 and August 2021 (NL7193).

## 2.1 | Study population

Consecutive adult allo-HSCT recipients who were referred by their hematologist for routine posttransplantation vaccinations were asked to participate in this study. According to the local hospital protocol, vaccinations are routinely started between 4 and 6 months after allo-HSCT,[2,9] but a delayed start was not considered an exclusion criterion.

Exclusion criteria were: diagnosis of a primary immune deficiency disorder, any hemophilic disorder precluding intramuscular vaccination, allergy to any of the components of the pneumococcal vaccines, donor lymphocyte infusion <28 days prior, pregnancy and not being able or willing to consent.

## 2.2 | Allo-HSCT procedure

All participants had received an allo-HCST with either myeloablative or nonmyeloablative conditioning regimens. In most cases, granulocyte colony-stimulating factor mobilized stem cells grafts were used, containing 4–10 × 10^6 CD34+ cells/kg. Prophylaxis for GvHD consisted of cyclosporine A and/or tacrolimus, dosed according to plasma concentrations, and mycophenolate mofetil 15/mg/kg. In the absence of GvHD, this prophylaxis was tapered according to local hospital protocol, with the timing depending on the type of transplant. The presence of GvHD at the baseline was graded and recorded by the treating hematologist during routine visits, based on international consensus.[10,11] The maximum grade of GvHD scored within 2 weeks from enrollment was recorded. When a patient without GvHD at baseline developed GvHD within 2 weeks after the first vaccination, this was also recorded as the presence of baseline GvHD.

## 2.3 | Sample size

The power calculation was based on expected differences in seroprotection rates after vaccination, between allo-HSCT recipients with and without immunosuppressive therapy at the baseline. Based on previous studies, we expected that in our population, no more than 60% of patients with immunosuppressive therapy and 90% without immunosuppressive therapy would seroconvert.[2,5] This difference would be detected with 80% power with a sample size of 36 in each group, using a Fisher's exact test with a 0.05 two-sided significance level. To compensate for an expected 10% drop out, we planned to recruit 40 individuals per group.

## 2.4 | Study procedures

An overview of study procedures is provided in Figure S1. PCV13 or Prevenar13® (Pfizer) includes purified capsular polysaccharide of 13 serotypes of *Streptococcus pneumoniae* (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) conjugated to a nontoxic variant of diphtheria toxin known as CRM197. PPSV23 or Pneumovax23® (Merck Sharp & Dohme) contains purified capsular polysaccharide of 23 serotypes (1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, and 33F). Participants concomitantly received other routine vaccinations that are part of the revaccination program following allo-HCST (Table S1). All vaccines were administered intramuscularly. Prior to the first vaccination (T0), we collected baseline clinical and laboratory data (including serum hemoglobin level, leukocytes, thrombocytes, serum IgG level, and absolute counts of lymphocytes and subsets of CD3/CD4/CD8/NK/B cells). Serum samples collected at baseline and at 4, 8, 10, and 12 months after

enrollment were frozen at −80°C until further analysis. Serotype-specific pneumococcal IgG serum concentrations were measured using a 26-plex multiplex immunoassay (Luminex technology) that includes all 24 vaccine serotypes of PCV13/PPSV23 combined, as well as nonvaccine serotypes 12A and 45 as controls. Samples were diluted in 5% antibody depleted human serum and 15 μg/ml pneumococcal cell wall polysaccharide and measured on a Magpix (Merck Millipore). Specific antibody concentrations were calculated based on the 007sp reference serum,[12-14] except for control serotypes 12A and 45 (arbitrary units assigned). After each vaccination, participants were asked to record solicited adverse events (AEs) through an online questionnaire. Serious and unsolicited AEs were recorded throughout the study period.

## 2.5 | Outcomes and analysis

The primary outcome of this study was the overall seroprotection rate 2 months after the full vaccination schedule (month 12), defined as the proportion of patients with a postimmunization IgG concentration of ≥1.3 μg/ml for at least 70% (17/24) of the serotypes of PCV13/PPSV23 combined, in allo-HSCT recipients with or without immunosuppressive therapy at baseline. Systemic steroids below <10 mg/day and a <700 mg cumulative dose were not considered as immunosuppressive therapy.

Defining an adequate response to pneumococcal vaccination is complex because consensus on the correlates of protection is lacking. The cut-off value of ≥0.35 μg/ml recommended by WHO is based on clinical studies in children using PCV7.[15] Prior research has shown that adults often have baseline antibody levels exceeding 0.35 μg/ml.[12,16-18] In addition, a recent study showed that the WHO cut-off, even in children, might lead to an over-estimation of protection for several serotypes.[19] Therefore, we used the more conservative correlate of protection of ≥1.3 μg/ml for 70% of serotypes proposed by the American Association of Allergy Asthma and Immunology (AAAAI).[16-18] To be able to compare our results to relevant prior studies,[2,5] the primary outcome and significant predictors for the primary outcome were also provided for the WHO cut-off.

Secondary outcomes were: (1) Seroprotection rates for each individual serotype and for the groups of serotypes unique to PCV13(1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 19A, 19F, 18C, and 23F) and PPVS23-unique serotypes (2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20, 22F, 33F); (2) serotype-specific geometric mean concentrations (GMC) of IgG and geometric mean fold rises (GMFR) for all 24 serotypes compared to baseline; (3) dynamics of seroprotection rates and GMCs over time; and (4) clinical and laboratory predictors for the primary outcome.

For all analyses, we used SPSS (IBM, Chicago, Illinois) version 25.0 or higher. Antibody concentrations were analyzed on a log-transformed scale and presented as GMC. The Kolmogorov–Smirnov test was used for testing the normal distribution. To detect changes in serotype-specific protection rates and GMC of IgG over time, generalized linear mixed models (GLMM, covariance structure first order autoregression) including the variables "time point" and "medication

group at baseline" were used. To identify predictors for the primary outcome as well as seroprotection by the WHO definition, a multivariable logistic regression analysis was performed including sex and age as fixed variables and using stepwise backward selection based on likelihood ratio and $p < .05$ for the following predefined variables: BMI, Charlson comorbidity index, type of allo-HSCT conditioning regimen (myeloablative or reduced intensity), receipt of post-transplantation cyclophosphamide, receipt of pretransplantation antithymocyte globulin, presence of acute or chronic GVHD at baseline, use of immunosuppressive medication at baseline (such as systemic corticosteroids, calcineurin inhibitors, mycophenolate mofetil), use of any immunosuppressive agents for GvHD at 8-months follow-up, time since allo-HSCT, baseline levels of IgG, hemoglobin, and lymphocytes and subsets. Interaction with variables age and sex was explored for all variables in the final model and included in the model if statistically significant. We used a two-sided alpha level of 0.05 for significance of statistical tests. Missing data were excluded from analysis. All analyses were done per protocol; all participants with two or more serum samples collected were included in the final analysis.

## 2.6 | Ethical considerations

The study was conducted according to the principles of the Declaration of Helsinki and in accordance with the Medical Research Involving Human Subjects Act (WMO). The research ethics committee of the Amsterdam UMC, Location AMC in 2018, provided ethical clearance (NL65687.018.18). All participants provided written informed consent for the study.

## 3 | RESULTS

Between August 2018 and July 2020, 93 allo-HSCT recipients were included in the study, of which 89 were included in the final analysis (Figure S2, Table S2). Briefly, allo-HSCT recipients using immunosuppressive therapy at baseline had less often received posttransplantation cyclophosphamide, had more often received antithymocyte globulin, were more often diagnosed with acute GvHD and had lower numbers of CD3, CD4, CD8, NK, and B-cells compared to patients not using immunosuppressive agents at the baseline. In addition, they were more often receiving IVIG at baseline, but only one had protective prevaccination pneumococcal antibody levels.

## 3.1 | Seroprotection rates

Twelve months after initiation of the vaccination schedule, 33% (26/78), 67% (50/78), and 19.2% (15/78) of HSCT recipients had achieved seroprotection against the serotypes of PCV13/PPSV23 combined, PCV13 serotypes, and serotypes exclusive to PPSV23, respectively (Figure 1; Table S3). Seroprotection rates using the WHO
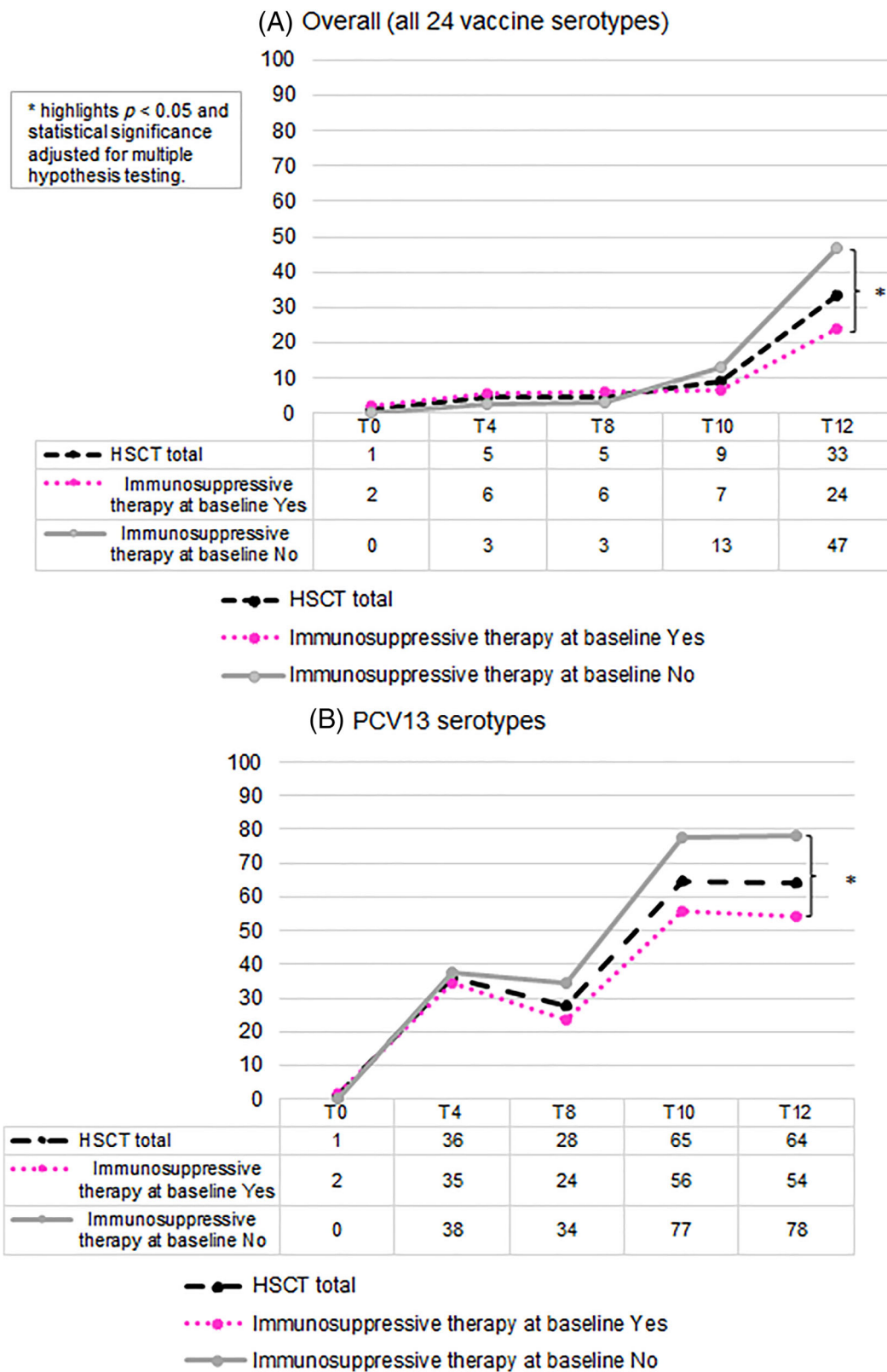
## (A) Overall (all 24 vaccine serotypes)

* highlights $p < 0.05$ and statistical significance adjusted for multiple hypothesis testing.

| | T0 | T4 | T8 | T10 | T12 |
|---|---|---|---|---|---|
| – ◆ – HSCT total | 1 | 5 | 5 | 9 | 33 |
| ⋯●⋯ Immunosuppressive therapy at baseline Yes | 2 | 6 | 6 | 7 | 24 |
| —○— Immunosuppressive therapy at baseline No | 0 | 3 | 3 | 13 | 47 |

– ◆ – HSCT total

⋯●⋯ Immunosuppressive therapy at baseline Yes

—○— Immunosuppressive therapy at baseline No

## (B) PCV13 serotypes

| | T0 | T4 | T8 | T10 | T12 |
|---|---|---|---|---|---|
| – ◆ – HSCT total | 1 | 36 | 28 | 65 | 64 |
| ⋯●⋯ Immunosuppressive therapy at baseline Yes | 2 | 35 | 24 | 56 | 54 |
| —○— Immunosuppressive therapy at baseline No | 0 | 38 | 34 | 77 | 78 |

– ◆ – HSCT total

⋯●⋯ Immunosuppressive therapy at baseline Yes

—○— Immunosuppressive therapy at baseline No

**FIGURE 1**    Overall seroprotection rate over time (A) and seroprotection rate for serotypes included in the 13-valent pneumococcal conjugate vaccine (B). HSCT, hematopoietic stem cell transplantation; PCV13, 13-valent pneumococcal conjugate vaccine; T, time point in months from first vaccine [Color figure can be viewed at wileyonlinelibrary.com]

cut-off were higher: 74%, 86%, and 50% for these three groups, respectively (Table S4). Patients treated with immunosuppressive agents at baseline were less likely to achieve overall seroprotection (OR 0.36; 95% CI 0.14–0.94) and protection against PCV13 serotypes (OR 0.33; 95% CI 0.12–0.92) at month 12 (Table 1), and this was also observed for the WHO cut-off (Table S4).

**TABLE 1** Factors associated with seroprotection 12 months after enrollment (univariable regression)

| Factor | Overall seroprotection rate (%) | Odds ratio (95% CI) | Seroprotection rate PCV13 (%) | Odds ratio (95% CI) | Seroprotection rate PPSV23-non PCV13 (%) | Odds ratio (95% CI) |
|---|---|---|---|---|---|---|
| Males | **33** | ref | 63 | ref | **20** | ref |
| Females | 34 | 1.1 (0.41–2.8) | 66 | 1.1 (0.44–2.9) | 19 | 0.95 (0.30–3.0) |
| Age group 18–49 | 30 | 0.84 (0.27–2.6) | 74 | 2.5 (0.82–7.7) | 22 | 1.4 (0.38–5.4) |
| Age group 50–59 | 38 | 1.2 (0.39–3.9) | 67 | 1.8 (0.55–5.6) | 19 | 1.2 (0.28–5.0) |
| Age group 60–70 | 33 | ref | 53 | ref | 17 | ref |
| BMI | NA | 1.04 (0.96–1.13) | NA | 0.98 (0.91–1.1) | NA | 1.1 (0.99–1.3) |
| Time since HSCT | NA | **1.0 (0.98–1.0)** | NA | **1.0 (0.98–1.0)** | NA | **1.0 (0.97–1.0)** |
| ≤6 months | 37 | ref | 67 | ref | 19 | ref |
| >6 months | 25 | 0.57 (0.19–1.7) | 58 | 0.70 (0.26–1.9) | 21 | 1.2 (0.35–3.8) |
| Myeloablative conditioning regimen | **16** | ref | 60 | ref | **4.0** | ref |
| Reduced intensity conditioning regimen | **42** | **3.7 (1.1–12.4)** | 66 | 1.3 (0.49–3.5) | **26** | **8.8 (1.1–69)** |
| Posttransplantation cyclophosfamide | | | | | | |
| Yes | 36 | ref | 66 | ref | 26 | ref |
| No | 29 | 0.72 (0.27–1.9) | 61 | 0.82 (0.32–2.1) | 9.7 | 0.31 (0.80–1.2) |
| No ATG in conditioning regimen | 35 | ref | 67 | ref | 17 | ref |
| ATG in conditioning regimen | 31 | 0.84 (0.31–2.3) | 58 | 0.66 (0.25–1.7) | 23 | 1.4 (0.45–4.6) |
| GVHD at baseline | 27 | 0.58 (0.22–1.5) | 54 | 0.43 (0.17–1.1) | 19 | 0.96 (0.31–3.0) |
| No GVHD at baseline | 39 | ref | 73 | ref | 20 | ref |
| Sibling donor | 32 | ref | 56 | ref | 16 | ref |
| Matched unrelated donor | 43 | 1.5 90.56–4.5) | 71 | 2.0 (0.70–5.5) | 26 | 1.9 (0.52–6.6) |
| Cord blood donor | 0 | NP$^a$, p-value $\chi^2=0.07$ | 50 | 0.79 (0.10–6.5) | 0 | NP p-value $\chi^2=0.25$ |
| Haplo-identical donor | 0 | | 57 | 1.1 (0.19–5.7) | 0 | |
| Use of immunosuppressive therapy at baseline | 24 | **0.36 (0.14–0.94)** | 54 | **0.33 (0.12–0.92)** | 13 | 0.38 (0.12–1.22) |
| No immunosuppressive therapy at baseline | 47 | **ref** | **78** | ref | 28 | ref |
| Steroids use at baseline | 25 | 0.62 (0.15–2.5) | 42 | 0.33 (0.10–1.2) | 8 | 0.34 (0.04–2.8) |
| Calcineurin inhibitor use at baseline | 26 | 0.59 (0.21–1.7) | 59 | 0.73 (0.28–1.9) | 19 | 0.93 (0.28–3.1) |
| Mycophenolic acid use at baseline | 25 | 0.64 (0.12–3.4) | 50 | 0.52 (0.12–2.3) | 13 | 0.57 (0.10–5.0) |
| Immunosuppressive therapy at month 8 | **16** | **0.22 (0.07–0.67)** | 53 | 0.45 (0.17–1.15) | 28 | **0.17 (0.04–0.81)** |

**TABLE 1** (Continued)

| Factor | Overall seroprotection rate (%) | Odds ratio (95% CI) | Seroprotection rate PCV13 (%) | Odds ratio (95% CI) | Seroprotection rate PPSV23-non PCV13 (%) | Odds ratio (95% CI) |
|---|---|---|---|---|---|---|
| No immunosuppressive therapy at month 8 | 46 | ref | 72 | ref | 6.3 | ref |
| Lymphocyte count at baseline <1.00 × 10⁹/L | 23 | ref | 48 | ref | 13 | ref |
| Lymphocyte count at baseline ≥1.00 × 10⁹/L | 40 | 2.32 (0.84–6.5) | 75 | **3.11 (1.2–8.1)** | 24 | 2.1 (0.59–7.2) |
| IgG level at baseline <6 g/L | 31 | 0.81 (0.29–2.3) | 77 | 2.2 (0.74–6.43) | 7.7 | 0.25 (0.05–1.2) |
| IgG level at baseline >6 g/L | 35 | ref | 60 | ref | 25 | ref |
| Hemoglobin level at baseline | NA | 1.24 (0.76–2.0) | NA | 1.3 (0.82–2.1) | NA | 1.34 (0.72–2.5) |
| CD4 count <200 at baseline cells/mm³ | 27 | ref | 50 | ref | 15 | ref |
| CD4 count >200 at baseline cells/mm³ | 37 | 1.6 (0.56–4.4) | 71 | 2.5 (0.93–6.5) | 21 | 1.5 (0.42–5.2) |
| B-cells <100 cells/mm³ at baseline | 26 | ref | 54 | ref | 8.6 | ref |
| B cells >100 at baseline cells/mm³ | 39 | 1.8 (0.69–4.9) | 73 | 2.3 (0.88–6.0) | 27 | 3.91 (1.0–15) |
| NK cells/mm³ at baseline | NA | 1.0 (1.0–1.0) | | 1.0 (1.0–1.0) | | 1.0 (1.0–1.0) |
| Asthma/COPD or other pulmonary disease | 33 | ref | 64 | ref | 18 | ref |
| No pulmonary disease | 33 | 1.00 (0.17–5.9) | 67 | 1.1 (0.19–6.6) | 33 | 2.3 (0.38–13) |
| Impaired kidney function (eGFR <60) | 34 | ref | 63 | ref | 21 | ref |
| Normal kidney function (eGFR ≥60) | 31 | 0.89 (0.27–3.9) | 69 | 1.3 (0.40–4.2) | 13 | 0.54 (0.11–2.7) |
| Charlson CoCo-morbidity Index | NA | 1.1 (0.80–1.6) | NA | 0.78 (0.55–1.1) | NA | 1.1 (0.70–1.6) |

*Note: N = 78.*

Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate; GvHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; PCV13, 13-valent pneumococcal conjugate vaccine; PPSV23, 23-valent pneumococcal polysaccharide vaccine.

[a]Not possible to calculate odds ratio and associated 95% confidence interval; therefore, *p*-value from $\chi^2$ test is provided.

Bold highlights indicate statistical significance (p-value <0.05).

The seroprotection rates differed widely for the individual serotypes (Table S5). At baseline, seroprotection rates for individual vaccine serotypes ranged between zero (PPSV23 exclusive serotype 12F) and 42% (PCV13 serotype 14). At month 12, seroprotection rates ranged between 15% (serotype 12F) and 94% (serotype 14). The seroprotection rates for individual serotypes increased significantly over time for all serotypes except 12F, both for patients with and without immunosuppressive therapy. Patients without immunosuppressive therapy at baseline, in comparison to those with, had significantly higher seroprotection over all time points against serotypes 1, 4, 9V (PCV13 serotypes), 9N, and 15B (PPSV23 exclusive serotypes). In addition, a significant interaction between time point and immunosuppressive therapy at baseline was observed for serotypes 7F, 14, 18C, 19A, and 19F (all PCV13 serotypes), indicating that the effect of immunosuppressive therapy on seroprotection was not the same for each time point (Table S5).

## 3.2 | Predictors of seroconversion at month 12

In the univariable analysis, receipt of a reduced intensity instead of a myeloablative conditioning regimen and a shorter time between HSCT and vaccination were positively associated with seroprotection against the serotypes of PCV13/PPSV23 combined, as well as against PCV13 serotypes and serotypes exclusive to PPSV23 (Table 1). In addition, a lymphocyte count above $1.00 \times 10^9$/L was positively associated with seroprotection against PCV13 serotypes and serotypes exclusive to PPSV23 (but not serotypes of PCV13/PPSV23 combined). Use of immunosuppressive medication at 8 months follow-up was negatively associated with seroprotection, both against the serotypes of PCV13/PPSV23 combined and the serotypes exclusive to PPSV23 (Table 1).

A multivariable logistic regression analysis showed that immunosuppressive therapy at baseline and treatment with a reduced intensity conditioning regimen were statistically significant predictors of overall seroprotection at T12 (Table 2). In addition, we observed a statistically significant effect of the interaction between sex and immunosuppressive therapy at baseline. Exploration revealed that seroprotection at T12 was significantly impaired in males who were treated with immunosuppressive medication compared to males who were not (overall seroprotection rate 15% versus 58% OR 0.11; 95% CI 0.02–0.46), while this impairment was not seen in females (seroprotection rate 38% versus 30% OR 1.01; 95% CI 0.20–5.04) (Table 3).

When predictors for seroprotection were analyzed by the WHO cut-off, receipt of a reduced intensity conditioning regimen was also a significant positive predictor, while the use of mycophenolate mofetil at baseline and any immunosuppressive drug at T8 significantly impaired seroprotection (Table 4).

## 3.3 | Antibody concentrations and dynamics over time

At baseline, geometric mean IgG concentrations of all vaccine serotypes were below the cut-off of 1.3 μg/ml (Figure 2; Table S6). At

**TABLE 2** Predictors of overall seroprotection (stringent cut-off) at month 12 (multivariable regression analysis of 78 patients)

| Factor | Adjusted OR | 95% CI |
|---|---|---|
| Sex (ref male) | 0.30 | 0.05–1.69 |
| Age at baseline | 1.0 | 0.95–1.0 |
| Immunosuppressive therapy at baseline (ref no therapy) | **0.01** | **0–0.32** |
| Reduced intensity conditioning regimen | **4.8** | **1.2–19** |
| Interaction immunosuppressive therapy at baseline * sex | **9.6** | **1.1–86** |

Bold highlights indicate statistical significance (p-value <0.05).

**TABLE 3** Predictors of overall seroprotection at month 12 (stringent cut-off): variables sex, immunosuppressive therapy at baseline and interaction term replaced by a single composed variable

| Factor | Adjusted OR | 95% CI |
|---|---|---|
| Age at baseline | 1.0 | 0.95–1.0 |
| Reduced intensity conditioning regimen | **4.8** | **1.2–19** |
| Males sex without immunosuppressive therapy | Ref | |
| Female sex with immunosuppressive therapy | 0.31 | 0.07–1.3 |
| Male sex with immunosuppressive therapy | **0.11** | **0.02–0.46** |
| Female sex without immunosuppressive therapy | 0.30 | 0.06–1.7 |

Bold highlights indicate statistical significance (p-value <0.05).

month 4, following the three PCV13 priming vaccinations, antibodies against 2/13 PCV13 serotypes increased to levels above the cut-off irrespective of the use of immunosuppressive therapy, followed by a decrease at month 8 (1/13 serotypes above the cut-off). The PCV13 booster dose led to an increase in antibody concentrations at T10, exceeding the cut-off for all 13 serotypes except serotype 3. The PPSV23 dose (T10) did not further increase antibody concentrations of any of the PCV13 serotypes. At T12, GMFRs of PCV13 serotypes ranged between 4.3 (serotype 19A) and 158 (serotype 4) compared to baseline (Figure 2A; Table S6).

In comparison to PCV13 serotypes, antibody concentrations against serotypes exclusive to PPSV23 were generally lower and remained unchanged and below any protective cut-off until T12. After completion of the full vaccination schedule, only serotype 2 elicited antibody concentrations above 1.3 μg/ml, irrespective of the use of immunosuppressive agents. At T12, GMFRs of PPSV23 serotypes compared to baseline ranged between 1.5 (serotype 15B) and 25 (serotype 9N) (Figure 2B; Table S6).

Over time, antibody concentrations against all 24 vaccine serotypes increased significantly. Antibody concentrations were significantly lower for PCV13 serotypes 1 and 7F and for PPSV23-exclusive serotype 2, in patients who used immunosuppressive agents at

**TABLE 4** Predictors of overall seroprotection (WHO cut-off) at month 12 (multivariable regression analysis of 78 patients)

| Factor | Adjusted OR | 95% CI |
|---|---|---|
| Sex (ref male) | 0.88 | 0.25–3.0 |
| Age at baseline | 0.98 | 0.93–1.0 |
| Reduced intensity conditioning regimen | **1.9** | **1.2–3.2** |
| Use of mycophenolate mofetil at baseline | **0.33** | **0.14–0.78** |
| Immunosuppressive therapy at month 8 | **0.56** | **0.37–0.87** |

Bold highlights indicate statistical significance (p-value <0.05).

baseline, but higher for PPSV23-exclusive serotypes 15B and 17F, which corresponded with higher baseline antibody concentrations for these serotypes (Figure 2A–C; Table S4). In addition, a significant interaction between time and immunosuppressive therapy at the baseline was observed for all serotypes except PPSV23 serotypes 8, 10A, 11A, 12F, 20, 22F, 33F and the nonvaccine serotypes 12A and 45, with a larger effect of immunosuppressive therapy on the IgG concentration at T10 and T12 for most serotypes (Figure 2A–C; Table S6).

## 3.4 | Safety

During the study, 14 serious SAEs occurred in 12 participants, none of which was related to any of the vaccinations. In total, 25 solicited adverse events (AEs) occurred in 15 participants (17%). The most commonly reported solicited AEs were injection site reactions (56%), fever (36%), musculoskeletal symptoms (32%), rash (24%), and head-ache or fatigue (12%). Most solicited AEs were mild or moderate (92%), and all patients recovered completely. In addition, 34 unsolicited AEs occurred in 26 individuals (29%), of which 80% were mild or moderate. Most reported unsolicited AEs were worsen-ing or new diagnosis of GVHD ($n = 12$); infectious complications ($n = 7$); or relapse of the hematological disease ($n = 4$). No episodes of (invasive) pneumococcal disease occurred during the study period.

## 4 | DISCUSSION

In this study, we determined the immunogenicity of a 5-dose pneu-mococcal vaccination schedule in adult allo-HSCT recipients with and without immunosuppressive therapy at baseline. Although the vacci-nation schedule was safe and well tolerated, overall seroprotection against the 24 vaccine serotypes was disappointing (33%), especially for patients using immunosuppressive therapy at baseline (24%). Much higher seroprotection rates following one dose of PCV13 followed by PPSV23 are achieved in healthy individuals (81%), and otherwise immunocompromised individuals such as IBD patients using immunosuppressive medication (59% or HIV patients (57%).[10,20] However, this low overall seroprotection rate was mainly caused by a

poor immunogenicity of the serotypes exclusive to PPSV23 (seroprotection rate 19%). The response to PCV13 was much better, with the majority of allo-HSCT recipients achieving seroprotection at month 12 (seroprotection rate 67%). In addition, GMFRs of IgG con-centration compared to baseline were above 4.0 for all PCV13 sero-types, independent of the use of immunosuppressive therapy at baseline, indicating that vaccination with PCV13 is immunogenic in allo-HSCT recipients. Previous studies investigating the immunogenic-ity of pneumococcal vaccines among allo-HSCT recipients focused on the immunogenicity of PCV serotypes and used less conservative cor-relates of protection than we did in the present study.[2,5,21,22] When we calculated SPRs by the lower WHO cut-off of 0.35 μg/ml, the response to PCV13 serotypes in our study was 86% which corre-sponds with seroprotection rates reported by others,[5] as well as a prior study done by our group, in which vaccinations were started much later after allo-HSCT, but only three doses of PCV13 were administered.[2] For serotypes exclusive to PPSV23 however, we report much lower response rates. Apart from the differences in the priming and booster schedule for both vaccines, this could be related to the PPSV23 vaccine being more dependent on a mature memory B-cell compartment to evoke an antibody response.[23,24] This would explain why patients who received a reduced intensity conditioning regimen, where memory B-cells and long-lived plasma cells survive longer, or may not be replaced at all, had a significantly higher SPR against the serotypes of PCV13/PPSV23 combined, and against the serotypes exclusive to PPSV23.[24,25] These findings could implicate that the recently approved 20-valent PCV[26] and the 24-valent pneumococcal vaccine candidate ASP3772,[27] both T-cell dependent, may be more suitable for allo-HSCT recipients compared to a vaccination schedule combining PCV13 and PPSV23.[23,24,28] Therefore, comparative studies investigating the immunogenicity and cost-effectiveness of the novel higher-valent T-cell dependent pneumococcal vaccines among allo-HSCT patients are much awaited.

Alternatively, to improve the response to PPSV23 serotypes, the PPSV23 dose could be postponed, as serologic response rates to PPSV23 serotypes were higher in a study in which PPSV23 was administered >22 months after allo-HSCT.[2] However, this strategy would leave allo-HSCT recipients unprotected against the most com-monly circulating pneumococcal serotypes to date.[6–8]

Our finding that specifically in males, the use of immunosuppres-sive agents at baseline significantly impaired the immunogenicity of the investigated vaccination schedule and had not been previously reported. Sex-based disparities in vaccination responses are however well-known phenomena[29,30] and are probably related to the immune-enhancing properties of estrogen and progesterone and the immuno-suppressive effects of testosterone.[31]

One of the dogmas regarding vaccination in allo-HSCT recipients is that the presence of acute[32] or chronic[33,34] GvHD is associated with impaired responses to vaccination. The multivariable analyses that we report here showed that the use of immunosuppressants was a better predictor of vaccination response than the presence of acute or chronic GvHD, suggesting that it is the immunosuppressive therapy to prevent or treat GvHD rather than GvHD itself that reduces
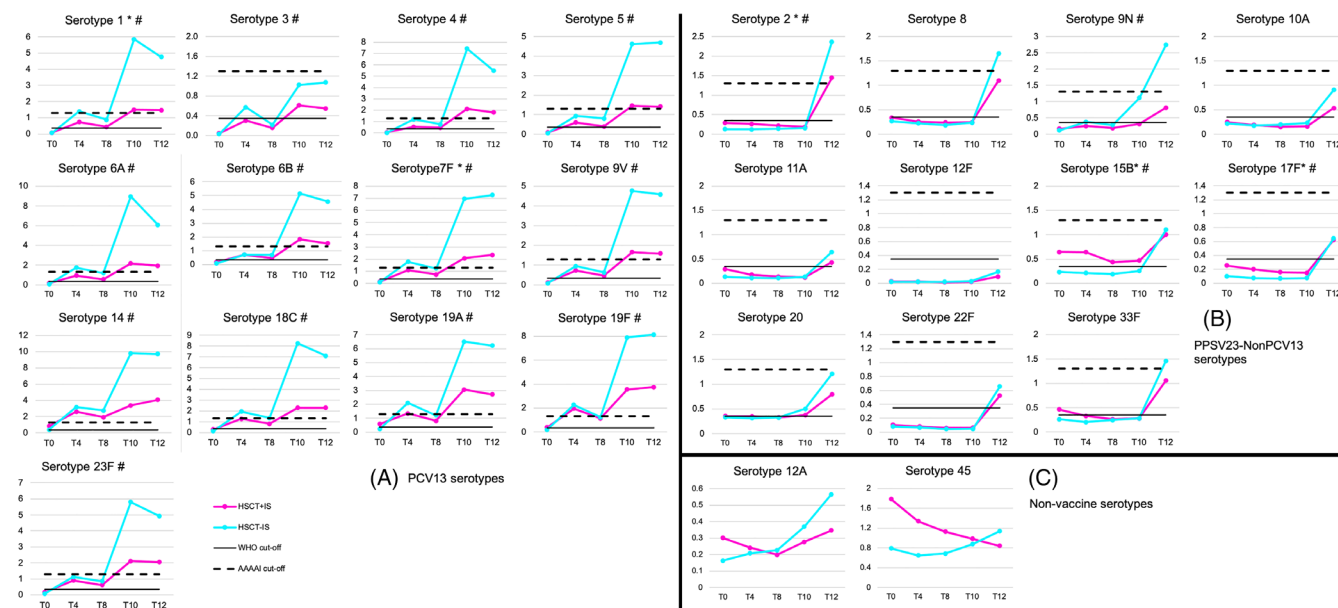
**FIGURE 2** Serotype-specific IgG levels in µg/ml over time for serotypes present in PCV13 (A), PPSV23 only (B), and two nonvaccine control serotypes (C). AAAAI, American Association of Allergy Asthma and Immunology (1.3 µg/ml); HSCT, hematopoietic stem cell transplantation; IS, immunosuppressive therapy at baseline; PCV13, 13-valent pneumococcal conjugate vaccine; PPSV23, 23-valent pneumococcal polysaccharide vaccine; T, time point in months from first vaccine; WHO, World Health Organization (0.35 µg/ml). *Statistically significant difference in GMC between patients with and without immunosuppressive therapy at baseline. #Statistically significant interaction between time point and immunosuppressive therapy at baseline [Color figure can be viewed at wileyonlinelibrary.com]

vaccination responsiveness. Most immunosuppressive agents are well known to cause a decrease in the numbers of lymphocytes, while this is less clear for (acute) GvHD itself. In the present study, we also observed that patients on immunosuppressive treatment had much lower lymphocyte counts compared with patients without immunosuppressive treatment, while rates of chronic GVHD were similar between the two groups. The effect of immunosuppressive therapy on vaccination responses was strong, suggesting it may be better to postpone pneumococcal vaccination in patients on immunosuppressants for a maximum of 3 months, especially in males and in patients who received myeloablative conditioning regimens. Postponing the vaccinations too long after allo-HSCT is not desirable because of the risk of early IPD among allo-HSCT recipients.[2,33] Importantly, vaccination responses should be monitored in these patients, as nonresponders may benefit from antimicrobial prophylaxis, on-demand antibiotics, or earlier booster vaccinations.

Currently, both European and U.S. guidelines recommend a booster dose of PCV13 6 months after the priming schedule only for HSCT recipients with GvHD, while for individuals without GvHD, a "booster" dose of PPSV23 is recommended.[3,4] Our results are in agreement with a prior study,[5] in which was shown that the booster dose of PCV13 administered 6 months after the priming schedule is important in order to maintain seroprotection for allo-HSCT recipients. In addition, there is evidence of reduced long-term protection when vaccinations are started earlier (4–6 months) after HSCT.[21] In our study, the fourth PCV13 (booster) dose at month 8 led to an additional 37% of participants being protected

against PCV13 serotypes, which is a substantial and clinically relevant increase. As no other studies investigated the currently recommended vaccination schedule of three doses of PCV13 plus a fourth vaccination with PPSV23, it remains uncertain if this would have caused a similar rise in seroprotection as a fourth vaccination with PCV13, which we studied. However, regarding the nature of the PPSV23 vaccine, which usually gives hyporesponse after PCV13 (as was seen in this study as well as in Cordonnier et al,), this seems unlikely.[5,28]

Despite the poor overall seroprotection rates for the serotypes exclusive to PPSV23 observed in this study, antibody concentrations of these serotypes did increase significantly for both patients with and without immunosuppressive therapy. Seroprotection rates and IgG levels against serotypes 8, 9N, 10A, and 22F (all exclusive to PPSV23), currently in the top 10 of most common serotypes causing IPD,[6–8] increased significantly in both patients with and without immunosuppressive therapy, with acceptable seroprotection rates for several of these common serotypes. In addition, no significant difference in the protection rates for PPSV23-unique serotypes between patients with and without immunosuppressive therapy was observed. Therefore, we suggest that until higher valent pneumococcal vaccines have been investigated, PPSV23 should be administered to all allo-HSCT recipients regardless of the use of immunosuppressive agents/GvHD.

A limitation of this study is that seroprotection rates are highly dependent on the chosen correlate of protection. As it has been suggested that the WHO cut-off of 0.35 µg/ml might be too low to reflect real-life protection for adults, we analyzed our data against

a higher, more conservative cut-off.[15–19] Adults generally have higher baseline and postvaccination antipneumococcal IgG concentrations for most serotypes compared to children, due to exposure to *S. pneumoniae* throughout life, with concentrations often exceeding the 0.35 μg/ml cut-off at baseline.[12,16–19] In addition, it has been suggested that adults need higher IgG levels than children for protection against *S. pneumoniae*, due to the shift from predominant IgG1 to predominant IgG2 responses, which occurs with age and may lead to lower opsonization and neutralization.[18] In reality, rather than one universal cut-off, each pneumococcal serotype has its own correlate of protection. However, as correlates of protection for PPSV23 serotypes are unknown, we decided to use the same cut-off for all pneumococcal serotypes. Only one study investigated the clinical effect of a 5-dose vaccination schedule on the incidence of IPD among allo-HSCT recipients and reported a strong decrease from 58.3/1000 to 5.6/1000 IPD cases. These observational data suggest a much higher effectiveness than what would be expected based on our immunogenicity data but could have been biased by a decrease of IPD incidence in the general population, as well as a higher rate of PCV serotypes at the time.[34] Alternatively, our chosen cut-off for seroprotection may have been too stringent for allo-HSCT recipients, whose immature immune system perhaps more resembles that of children than that of adults. Therefore, we have also provided the seroprotection rate and predictors of the vaccination response for the WHO cut-off.

A recent study reported that 50% of allo-HSCT recipients had lost immunity to PCV serotypes 5 years after vaccination and recommended monitoring antibody measurements from 24 months after vaccination.[35] We are currently investigating decay of antibody concentrations in the present cohort.

## 5 | CONCLUSION

The proposed 5-dose pneumococcal vaccination schedule is safe and shows acceptable immunogenicity for PCV13 serotypes, especially in allo-HSCT recipients without immunosuppressive therapy at baseline. As the response to serotypes exclusive to PPSV23 is poor, research into novel vaccination strategies with higher valent T-cell-dependent pneumococcal vaccines is needed. Until then, a booster dose of PCV13 as well as a dose of PPSV23 to broaden the spectrum of seroprotection should be recommended for all allo-HSCT recipients, regardless of the presence of GvHD.

## DATA AVAILABILITY STATEMENT
The datasets used during the current study are available from the corresponding author on reasonable request.

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## SUPPORTING INFORMATION

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