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Pneumococcal vaccine responses in elderly patients with multiple myeloma, Waldenstrom's macroglobulinemia, and monoclonal gammopathy of undetermined significance

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ABSTRACT

Background: Vaccination with the 23-valent pneumococcal polysaccharide vaccine (PPV) has been recommended for elderly patients with B cell malignancies and dysfunctions because of their enhanced susceptibility to pneumococcal infections. More recent recommendations advocate the use of conjugate pneumococcal vaccines.

Methods: We compared responses to single dose vaccination with either PPV or the 7-valent pneumococcal conjugate vaccine (PCV7) in fifty-six patients ⩾60 years with a diagnosis of multiple myeloma ($n = 24$), Waldenstrom's macroglobulinemia ($n = 15$) and the non-malignant B cell disorder monoclonal gammopathy of undetermined significance (MGUS) ($n = 17$), and 20 age-matched controls. Serum was collected prior to vaccination and 4–8 weeks later, and analyzed for IgG antibody levels to pneumococcal serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F by ELISA. Functional antibody activity towards pneumococcal serotypes 4 and 14 was measured using an opsonophagocytic killing assay (OPA).

Results: All patient groups had lower pre-vaccination IgG antibody and OPA titers to the investigated serotypes compared to the healthy controls. Following vaccination, myeloma patients responded with significant IgG titer increases to 1/7 serotypes and OPA titer increases to 1/2 serotypes. Corresponding IgG and OPA vaccine responses were 3/7 and 0/2 for Waldenstrom patients, 4/7 and 1/2 for MGUS patients, and 4/7 and 2/2 for the healthy controls, respectively. Notably high antibody levels without corresponding OPA titers were seen among a few myeloma patients indicating the presence of non-functional antibodies. Neither of the two vaccines elicited significantly higher serotype-specific IgG concentrations, OPA titers or antibody fold increases in any of the study groups. Hypogammaglobulinemia and ongoing chemotherapy were associated with poor vaccine responses in a multivariate analysis.

Conclusion: Our findings confirm that B cell malignancies and disorders among elderly patients are associated with suboptimal responses to pneumococcal vaccination. Single-dose PCV7 was not shown to be superior to PPV.

1. Introduction

Multiple myeloma (MM) and Waldenstrom's macroglobulinemia (WM) are hematological malignancies arising from plasma cells and lymphoplasmacytoid cells, respectively, and mainly afflict aged persons. Monoclonal gammopathy of undetermined significance (MGUS) is a related non-malignant disorder engaging B cells/plasma cells that also affects the elderly and can transform into malignant disease. Common to these B cell disorders are varying degrees of hypogammaglobulinemia and clonal overproduction of non-functional immunoglobulins called M-protein. Besides the inherent impaired humoral immunity characteristic of B cell

Abbreviations: PPV, 23-valent pneumococcal polysaccharide vaccine; PCV7, 7-valent pneumococcal conjugate vaccine; MGUS, monoclonal gammopathy of undetermined significance; IgG, immunoglobulin G; OPA, opsonophagocytic killing assay; MM, multiple myeloma; WM, Waldenstrom's macroglobulinemia; CRM_{197}, cross reactive material (CRM_{197}: mutant peptide related to diphtheria toxoid); CPS, cell wall polysaccharide; O-PLS, orthogonal partial least squares projections to latent structures; WBC, white blood cell count.

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malignancies, cellular immune functions may also be depressed by the disease itself, and further aggravated by chemotherapy and other immunosuppressive therapies used to treat it.

Multiple myeloma is strongly associated with infectious susceptibility, which is particularly pronounced during the first months after diagnosis. During this phase, infections with gram-positive bacteria, above all *Streptococcus pneumoniae*, predominate, and are primarily ascribable to diminished immunoglobulin synthesis [1,2]. A British study reported that 45% of early deaths (within 60 days of diagnosis) in MM patients were related to infections, mainly pneumonia and sepsis [3]. Although less studied, patients with WM or MGUS also have increased mortality due to pneumonia and sepsis [4,5]. A recent population-based Swedish study found a more than doubled risk of pneumonia in MGUS patients compared to matched controls [6].

Pneumococcal vaccination is generally recommended for patients with B cell malignancies [1,7]. The 23-valent pneumococcal polysaccharide vaccine (PPV) has been widely used in risk groups including healthy elderly. A recent meta-analysis estimated the PPV had a protective efficacy of 74% (95% confidence interval, 55–86%) against invasive pneumococcal disease in adults [8]. However, its effectiveness in elderly healthy individuals, as well as in MM patients has been debated [1,2,8,9]. Previous studies of MM patients have shown suboptimal antibody levels in 50–60% after vaccination with PPV [10,11]. We have documented that elderly MM patients have low anti-pneumococcal serum antibody levels [12].

Conjugated pneumococcal vaccines induce a T cell-dependent, possibly longer-lasting immune response, and may be more immunogenic than PPV not least in immunocompromised hosts. Regimens involving repeated doses of the 7-valent pneumococcal conjugated vaccine (PCV7) appear to be promising in hematopoietic stem cell transplant recipients [13–16], and vaccination schedules containing PCV7 followed by PPV have recently been recommended by the European Group for Blood and Marrow Transplantation as well as by the Centers for Disease Control and Prevention [7,17]. Non-transplanted MM patients have been routinely vaccinated with PPV in our region in the west of Sweden.

To our knowledge, no previous studies on pneumococcal vaccine responses in patients with WM or MGUS have been performed. The aims of this study were 1) to investigate the ability of elderly, non-stem cell transplanted patients with MM, WM or MGUS to mount an immune response to pneumococcal vaccination as measured by IgG antibody responses as well as by functional (opsonophagocytic) antibody activity, and 2) to compare the immunogenicity of PCV7 to PPV in single dosage in these patient categories.

2. Materials and methods

2.1 Study population

Patients aged $\geqslant$60 years with MM (n = 24), WM (n = 15) or MGUS (n = 17) were recruited to the study from the outpatient clinic of the Dept. of Hematology, Uddevalla Hospital, Sweden, between May 2008 and March 2009. WHO criteria were used to establish the hematologic diagnoses [18]. Exclusion criteria were previous or scheduled hematopoietic stem cell transplantation, pneumococcal vaccination or infection (septicemia, meningitis or pneumonia) less than a year prior to the study, and previous severe adverse reactions (angioedema or anaphylaxia) following any kind of vaccination. An age-matched control group without hematological disorders (n = 20) was recruited during the same period. The study participants were asked to fill in a questionnaire regarding previous vaccinations, ongoing medication and smoking habits. Written informed consent was obtained from all patients. The study was approved by the Regional Ethics Committee in Göteborg.

A total of 81 persons were primarily recruited to the study. Two individuals, one patient with WM and one control person, withdrew before the second visit. Three participants were later excluded due to protocol violations; one control person who was too young at the time of inclusion, two patients (one MM, one MGUS) with pneumonia within a year prior to inclusion.

2.2. Vaccination and sampling of study persons

All study persons were given a single intramuscular dose of pneumococcal vaccine in the upper arm. In each of the four study groups, subjects were randomized to receive 0.5 ml of either PCV7 (Prevenar; Pfizer) or PPV (Pneumo 23; Sanofi Pasteur). The PCV7 vaccine contained 2 μg of the respective polysaccharide of serotypes 4, 9V, 14, 18C, 19F and 23F, and 4 μg of serotype 6B, all individually conjugated to the diphtheria carrier protein CRM_{197}. The PPV vaccine contained 25 μg of each of the 23 capsular polysaccharide components. Blood samples were collected directly before and 4–8 weeks after vaccination. Serum was separated and stored at −20 °C until analyzed. Serious adverse events occurring during the study and up to three months after its termination were retrieved from the patients' records.

2.3. Laboratory assays

2.3.1. Pneumococcal enzyme-linked immunosorbent assay (ELISA)

Serum IgG antibodies to the seven pneumococcal serotypes (4, 6B, 9V, 14, 18C, 19F, and 23F), present in PCV7 as well as in PPV, were assayed at Statens Serum Institut, Copenhagen, Denmark, using an ELISA described in detail elsewhere [19] after cell wall (CPS, c-polysaccharide) adsorption. A local reference serum calibrated to the US standard reference serum 89-SF was used. Antibody concentrations were expressed as μg/ml.

2.3.2. Opsonophagocytosis

Functional antibody activity was measured by a modified two-fold multiplexed opsonophagocytosis assay (OPA) as previously described [20,21]. Pneumococcal strains of serotypes 4 and 14 resistant to optochin and streptomycin, respectively, were obtained from BEI Resources (Manassas, VA). Differentiated HL-60 cells (ATCC, Manassas, VA) were used as effector cells to phagocytose pneumococci in the presence of patient serum antibodies and baby rabbit complement (Invitrogen, Lidingö, Sweden). OPA titers were expressed as the reciprocal of the serum dilution causing 50% bacterial killing compared with the bacterial growth in the control wells without serum. The lowest OPA titer that could be determined was 4. In patients receiving antibiotics during the study (four MM patients, one WM patient on prophylactic cotrimoxazole, one MM patient on cloxacillin), the bactericidal effect of serum alone was compared to non-antibiotic treated patients, but no difference was seen. Sera from sixteen patients were analyzed at the National Institute for Health and Welfare, Helsinki, Finland. The remaining sera were analyzed at the Dept. of Clinical Bacteriology, Sahlgrenska University Hospital, Göteborg, Sweden.

2.4. Statistical analyses

Pneumococcal antibody and OPA titers were expressed as geometric means with 95% confidence intervals. Titers below the detection limit were set to 0.5 times this limit. Comparisons of geometric means before and after vaccination were done using the Wilcoxon signed rank test, between study groups using the Mann–Whitney U test. The proportions of subjects with IgG or

OPA titers above the cut-off levels were compared using Fisher's exact test. Graph Pad Prism 5.0 software was used (GraphPad, San Diego, CA). The multivariate analysis "Orthogonal partial least squares projections to latent structures" (O-PLS) was used to assess the influence of clinical parameters on vaccine responses with the aid of the SIMCA-P statistical programme (Umetrics, Umeå, Sweden) [22]. Data derived from the O-PLS models were further analyzed using the Spearman rank correlation test and the Mann–Whitney *U* test as appropriate. *P* values were two-sided. A significance level of $P < 0.05$ was used.

3. Results

3.1. Study population

Baseline characteristics of the 76 study persons are presented in Table 1. The median age did not differ significantly between the study groups. Hypogammaglobulinemia was seen in 92% of MM patients versus 40% of WM patients ($P = 0.0009$) and 53% of MGUS patients ($P = 0.008$). More than half of the MM patients (63%) had ongoing chemotherapy and/or immunomodulatory treatment at the time of vaccination, reflecting clinical reality.

3.2. ELISA

Pneumococcal IgG titers before and after vaccination were consistently the lowest in the MM patients. Moreover, a stepwise antibody pattern was seen with next-to-lowest titers among WM patients, followed by MGUS patients, and healthy controls, respectively (Fig. 1, Table 2). Two MM patients differed from the rest: one had very high pre-immune IgG titers (Fig. 1A and E), the other appeared to respond exceptionally well to vaccination (Fig. 1B and F).

Geometric mean IgG concentrations increased modestly after vaccination, ranging from no increase to a maximum fourfold increase (seen for serotype 18C in WM patients who received PCV7) (Table 2). Statistically significant IgG increases were seen for one out of seven serotypes among MM patients (18C), 3/7 serotypes among the WM patients (9V, 18C, 19F), and 4/7 serotypes among the MGUS patients and controls alike (9V, 14, 18C, 19F).

Baseline IgG antibody concentrations were similar for the PCV7 subgroups and the PPV subgroups for all seven pneumococcal serotypes (Table 2). Following vaccination, no significant differences were seen regarding absolute antibody titers (Table 2) or antibody fold increases (data not shown) between the vaccine types for any of the study groups or serotypes. Since the numbers of individuals in each study group were small, we also calculated combined antibody fold increases against all seven serotypes, and found significantly higher responses to PCV7 in the WM group (23% higher), and to PPV in the MGUS group (41% higher) (Table 3), although all fold increases were $\leqslant 2$. For the healthy controls, identical response rates were obtained for the two vaccine types (Table 3). As mentioned, two MM patients displayed very high antibody titers to all serotypes tested, one patient before, the other after vaccination. Both patients were on immunomodulatory treatment and had low serum IgG levels, and it was suspected that these results were falsely positive. When these individuals were excluded from the calculations, combined antibody fold increases were significantly higher to PCV7 than to PPV also in the MM group (1.79 vs. 1.60, $P = 0.04$).

Antibody levels of $\geqslant 0.5$ and $\geqslant 1.0$ μg/ml were chosen as points of reference in accordance with previous studies on immunosuppressed patients [16,23,24] since a true correlate of seroprotection in adults has not been defined. The proportions of subjects with serotype-specific (data not shown) or combined (Table 3) IgG antibody levels $\geqslant 0.5$ and $\geqslant 1.0$ μg/ml did not differ between the vaccine groups. In total, 63% of the MM patients, 73% of the WM patients, 88% of the MGUS patients, and 100% of the healthy controls had antibody levels $\geqslant 0.5$ μg/ml, and 38% (MM), 60% (WM), 65% (MGUS), and 80% (controls) had IgG $\geqslant 1.0$ μg/ml to all seven serotypes after vaccination.

Table 1
Study group characteristics.

	Multiple myeloma	Waldenstrom's macro-globulinemia	Monoclonal gammopathy of undetermined significance	Healthy controls	All groups
No. of subjects	24	15	17	20	76
Median age, years (range)	76 (62–88)	75 (62–88)	71 (60–80)	69 (61–83)	74 (60–88)
Female sex, *n*	12	9	11	11	43
Smokers, *n*	1	2	2	0	5
Previous pneumococcal vaccination, *n*	3	3	3	3	12
WBC median, $\times 10^9$ L^{-1} (range)[a]	4.3 (1.3–9.4)	7.1 (2.6–18)	5.9 (3.0–8.8)	6.6 (4.2–11.7)	-
S-IgG median, g/L (range)[b]	1.7 (0.1–8.0)	7.5 (1.0–11)	5.3 (0.3–11)	11 (8.2–19)	-
M-protein median, g/L (range)[c]	27 (0.7–49)	15 (3.0–28)	11 (0.5–28)	0 (0–0)	-
Hypogammaglobulinemia, s-IgG <6.1, *n*	22	6	9	0	37
Ongoing immunomodulatory therapy, *n*	15	1	0	0	16
Treatment regimens, *n*					
Melphalan + prednisone	5	0	0	0	5
Cyclophosphamide + dexamethasone	6	0	0	0	6
Pulse steroids	2	0	0	0	2
Thalidomide	1	0	0	0	1
Bortezomib[d]	2	0	0	0	2
Fludarabin	0	1	0	0	1
Treatment-naïve subjects, *n*	3	8	17	20	48
Ongoing antipyretic therapy[e], *n*	6	3	7	6	22
Given vaccine type, *n*					
PCV7[f]	12	8	8	9	37
PPV	12	7	9	11	39

[a] WBC, white blood cell count; reference level in blood = 3.5–8.8 $\times 10^9$ L^{-1}.
[b] S-IgG, serum immunoglobulin G; reference level in serum = 6.1–14.9 g/L.
[c] M-protein, monoclonal protein.
[d] One MM patient had a combination regimen of bortezomib, pulse steroids, and melphalan.
[e] Paracetamol or non-steroid anti-inflammatory drugs.
[f] PCV7, 7-valent pneumococcal conjugate vaccine; PPV, 23-valent pneumococcal polysaccharide vaccine.

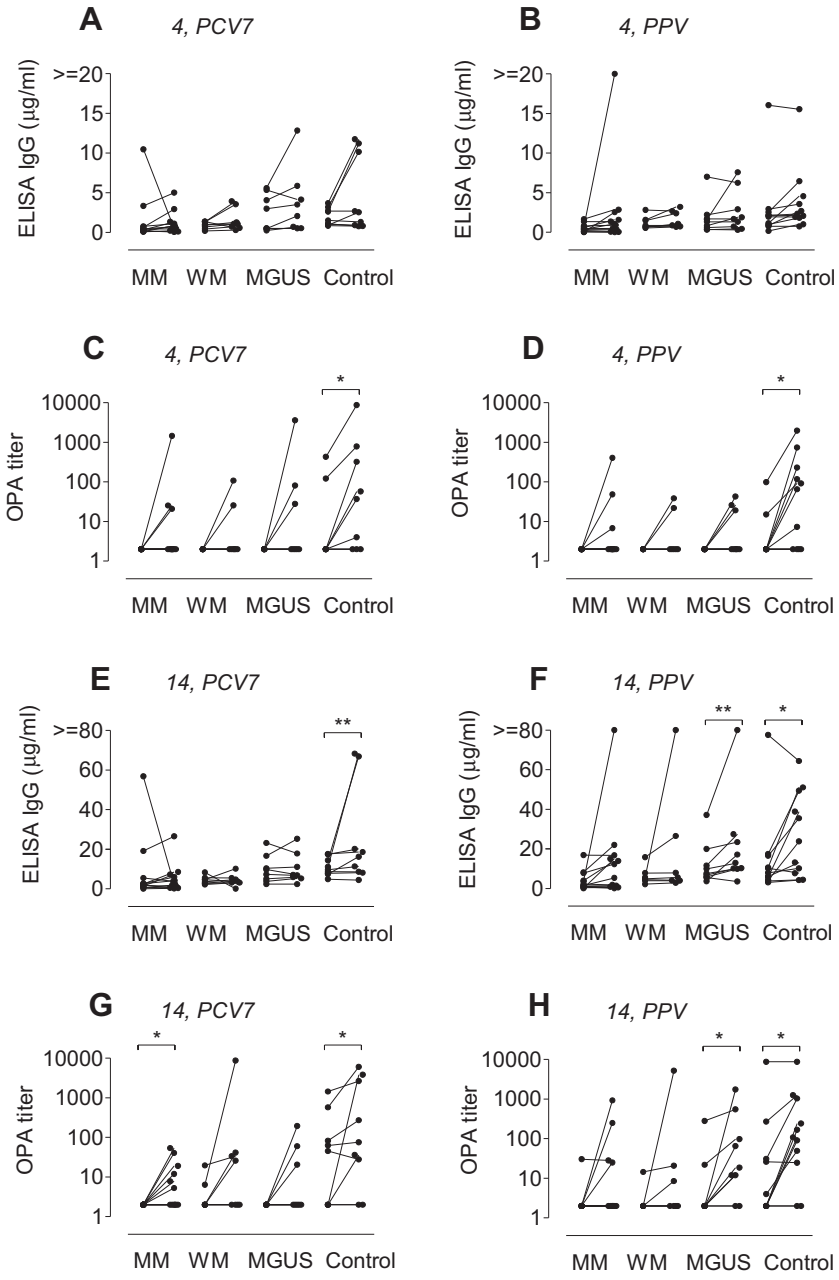

Fig. 1. Anti-pneumococcal IgG (ELISA) and opsonophagocytic antibody (OPA) titers before and 4–8 weeks after vaccination. (A and B), serotype 4, ELISA; (C and D), serotype 4, OPA; (E and F), serotype 14, ELISA; (G and H) serotype 14, OPA. (A, C, E, G) show PCV7 vaccinees, (B, D, F, H) show PPV vaccinees. Pre- and post-vaccination titers for each study person are shown. Note that Y values vary between the serotypes for the ELISA analyses. $^{*}P < 0.05$; $^{**}P < 0.01$ (Wilcoxon signed rank test).

3.3. Opsonophagocytosis

The opsonic assay confirmed the ELISA results regarding serotype 14, with the lowest OPA titers occurring in MM patients followed by WM, MGUS and healthy controls (Fig. 1, Table 4). For serotype 4, all three patient groups had poorer vaccine responses than the control group. The high ELISA IgG titers seen in two MM patients were not confirmed by the OPA results (Fig. 1).

Following vaccination, OPA titers against both pneumococcal serotypes increased in all study groups, but significant rises were only seen in MM patients who had received PCV7 (serotype 14), in MGUS patients who had received PPV (serotype 14), and in healthy controls given either vaccine (serotypes 4 and 14) (Table 4).

Baseline OPA titers did not differ significantly between the vaccine subgroups (Table 4). No obvious trend favoring either vaccine type could be discerned in any of the study groups. Hence, statistically significant increments in absolute OPA titers against serotype 14 were seen in MM patients in response to PCV7, in MGUS patients against serotype 14 after PPV, and to both serotypes following vaccination with either of the two vaccines in healthy controls (Table 4). No statistically significant differences were noted regarding the post-vaccination titers evoked by either of the vaccines, in any of the four study groups (Table 4). The same was true for fold increases of OPA titers after vaccination (Table 5). Between two- and ninefold OPA titer increments were noted, with the lowest elevations seen among the MM and WM subgroups (Table 5).

An OPA titer of 8 was chosen as point of reference in accordance with previous studies [25,26]. The proportions of patients with OPA titers $\geqslant 8$ were similar between the vaccine subgroups (Table 5). In total, 8% of the MM patients, 27% of the WM patients, 29% of

Table 2
Anti-pneumococcal IgG antibodies among PCV7 and PPV vaccinees, respectively, before and after vaccination.

Vaccine, study group	Timepoint	Geometric mean IgG concentration, μg/ml (95% confidence interval), by serotype						
		4	6B	9V	14	18C	19F	23F
PCV7								
MM	T0	0.44 (0.18–1.09)	3.58 (1.37–9.41)	0.66 (0.22–2.00)	2.40 (0.90–6.43)	0.52 (0.25–1.07)	1.82 (0.70–4.71)	1.13 (0.41–3.09)
	T1	0.61 (0.26–1.39)	3.79 (1.85–7.77)	0.72 (0.27–1.95)	2.49 (1.05–5.95)	0.98 (0.53–1.81)	2.72 (1.25–5.93)	1.49 (0.75–2.91)
WM	T0	0.77 (0.43–1.38)	4.47 (2.99–6.68)	1.18 (0.70–1.97)	3.99 (2.78–5.74)	0.82 (0.49–1.37)	2.76 (1.68–4.54)	1.99 (1.34–2.97)
	T1	1.12 (0.54–2.32)	5.69 (2.95–10.96)	2.10 (0.81–5.46)**	2.82 (0.87–9.10)	3.31 (0.86–12.34)*	5.89 (1.92–18.11)**	2.98 (1.56–5.70)
MGUS	T0	1.34 (0.45–3.95)	11.56 (5.29–25.26)	3.07 (1.29–7.24)	7.75 (4.12–14.60)	1.91 (0.81–4.49)	5.04 (1.99–12.79)	4.04 (1.67–9.79)
	T1	2.13 (0.78–5.84)	11.75 (5.51–25.07)	5.04 (1.64–15.54)**	8.09 (4.37–15.00)	3.11 (1.19–8.13)**	5.31 (2.10–13.41)	5.24 (1.57–17.51)
Controls	T0	1.68 (1.08–2.63)	8.27 (4.77–14.34)	3.32 (1.85–5.98)	10.22 (7.38–14.14)	2.21 (1.41–3.46)	7.12 (4.28–11.83)	3.69 (2.78–4.90)
	T1	2.66 (1.10–6.48)	8.28 (4.12–16.66)	7.77 (2.99–20.18)*	16.71 (8.24–33.90)*	8.18 (2.87–23.34)**	10.01 (4.65–21.57)	7.37 (2.74–19.78)
PPV								
MM	T0	0.31 (0.13–0.74)	2.55 (0.96–6.80)	0.66 (0.24–1.79)	1.86 (0.75–4.64)	0.49 (0.25–0.97)	1.36 (0.60–3.08)	0.83 (0.36–1.90)
	T1	0.65 (0.19–2.23)	4.23 (1.29–13.86)	1.36 (0.36–5.09)	4.67 (1.40–15.65)	1.15 (0.36–3.64)*	2.25 (0.67–7.58)	1.69 (0.46–6.23)
WM	T0	1.06 (0.61–1.83)	6.22 (3.25–11.91)	1.74 (1.03–2.95)	5.39 (3.07–9.47)	1.14 (0.52–2.47)	3.62 (2.18–6.02)	2.59 (1.68–3.98)
	T1	1.39 (0.76–2.55)	6.94 (4.26–11 28)	2.19 (1.18–4.07)	9.02 (2.85–28.52)	1.67 (0.60–4.63)	4.64 (2.48–8.71)	3.19 (2.14–4.76)
MGUS	T0	1.16 (0.56–2.41)	7.21 (3.07–16.91)	1.86 (0.85–4.07)	9.32 (5.40–16.08)	2.59 (1.83–3.65)	4.60 (2.71–7 .80)	3.24 (1.69–6.24)
	T1	1.56 (0.67–3.66)	8.24 (3.71–18.27)	4.02 (1.40–11.57)*	15.67 (7.58–32.34)**	7.45 (4.18–13.27)*	9.65 (4.91–18.98)*	6.55 (3.08–13.93)
Controls	T0	1.58 (0.75–3.30)	11.24 (6.74–18.73)	3.71 (2.09–6.58)	9.04 (4.90–16.69)	3.07 (1.51–6.21)	8.84 (5.34–14.63)	4.16 (2.61–6.65)
	T1	2.71 (1.54–4.78)	13.83 (9.31–20.52)	7.98 (3.83–16.62)**	18.81 (9.60–36.86)*	8.30 (4.08–16.86)**	14.86 (8.04–27.46)**	5.70 (3.57–9.10)

PCV7, 7-valent pneumococcal conjugate vaccine; PPV, 23-valent pneumococcal polysaccharide vaccine.
MM, multiple myeloma; WM, Waldenstrom's macroglobulinemia; MGUS, monoclonal gammopathy of unknown significance.
T0, baseline (*i. e*., before vaccination); T1, 4–8 weeks after vaccination.
*$P < 0.05$; **$P < 0.01$. Comparison of post vs. pre-vaccination antibody concentrations for each study group, respectively; Wilcoxon signed rank test.
P values for comparisons of vaccine subgroups (PCV7 vs. PPV; pre and post vaccination) are all >0.05 (Mann–Whitney *U* test).

Table 3
Number of subjects with anti-pneumococcal IgG titers ⩾0.5 and ⩾1.0 μg/ml to all seven investigated serotypes before and after vaccination with either PCV7 or PPV, and geometric means of antibody fold increases combined for all seven serotypes.

Study group	Time-point	IgG ⩾0.5 μg/ml (%)		IgG ⩾1.0 μg/ml (%)		IgG fold titer increase		
		PCV7	PPV	PCV7	PPV	PCV7	PPV	P
MM	T0	4 (33)	5 (42)	2 (17)	2 (17)			
	T1	7 (58)	8 (67)	2 (25)	6 (50)	1.29	2.03	0.97
WM	T0	6 (75)	6 (86)	2 (25)	2 (29)			
	T1	5 (63)	6 (86)	5 (63)	4 (57)	1.62	1.32	0.01
MGUS	T0	5 (63)	7 (78)	4 (50)	5 (56)			
	T1	8 (100)	7 (78)	5 (63)	6 (67)	1.30	1.83	0.03
Controls	T0	9 (100)	10 (91)	6 (67)	7 (64)			
	T1	9 (100)	11 (100)	6 (67)	10 (91)	1.81	1.79	0.45

PCV7, 7-valent pneumococcal conjugate vaccine; PPV, 23-valent pneumococcal polysaccharide vaccine.
MM, multiple myeloma; WM, Waldenstrom's macroglobulinemia; MGUS, monoclonal gammopathy of unknown significance.
T0, baseline (*i. e*., before vaccination); T1, 4–8 weeks after vaccination.
Numbers in parentheses indicate percent responders.
P values for comparisons of vaccine subgroups (PCV7 vs. PPV) regarding antibody titers ⩾0.5 and ⩾1.0 μg/ml are all >0.05 (Fisher's exact test).

the MGUS patients, and 55% of the healthy controls had OPA titers ⩾8 to both serotypes after vaccination.

3.4. Association of clinical variables with vaccine response

Data were analyzed by the multivariate technique of O-PLS to construct a model showing the relationship between response to vaccination as measured by post-vaccination OPA titers to serotype 14 and patient characteristics. The model had an explanatory power of 50% ($r^2 = 0.50$) and validity of 16% ($q^2 = 0.16$). According to the model, variables associated with good vaccine responses were high levels of serum IgG and WBC, and no previous chemotherapy. Corresponding univariate correlation analyses were significant only for IgG and WBC in the WM group (data not shown). Conversely, hypogammaglobulinemia, high levels of M-protein and ongoing therapy with cyclophosphamide and dexamethasone, or other immunomodulators were negatively associated with vaccine responses in the O-PLS model. This tendency was seen in univariate, group-specific analyses as well but did not reach statistical significance (data not shown). None of the nine MM and WM patients with serum IgG levels <1.0 g/L had a clear response to vaccination, *i.e*., OPA titers >8 to both serotype 4 and 14, compared to six of the remaining 30 patients. This difference did not quite reach statistical significance. No association was found between post-vaccination OPA titers and age, sex, smoking habits, previous pneumococcal vaccination, given vaccine type, ongoing antipyretic therapy, or interval (days) between vaccination and the follow-up serum sampling.

Table 4
Anti-pneumococcal functional antibody titers as measured by opsonophagocytic assay (OPA) among PCV7 and PPV vaccinees, respectively, before and after vaccination.

Vaccine, study group	Timepoint	Geometric mean OPA titer (95% confidence interval), by serotype		Vaccine, study group	Timepoint	Geometric mean OPA titer (95% confidence interval), by serotype	
		4	14			4	14
PCV7				*PPV*			
MM	T0	2.00 (2.00–2.00)	2.00 (2.00–2.00)	MM	T0	2.00 (2.00–2.00)	2.51 (1.52–4.13)
	T1	5.21 (1.45–18.69)	5.76 (2.58–12.88)*		T1	4.50 (1.52–13.29)	7.68 (1.91–30.82)
WM	T0	2.00 (2.00–2.00)	3.08 (1.51–6.27)	WM	T0	2.00 (2.00–2.00)	2.66 (1.33–5.31)
	T1	4.53 (1.23–16.73)	16.34 (1.45–184.2)		T1	4.30 (1.27–14.55)	10.57 (0.73–152.6)
MGUS	T0	2.00 (2.00–2.00)	2.00 (2.00–2.00)	MGUS	T0	2.00 (2.00–2.00)	4.52 (1.19–17.22)
	T1	11.27 (1.13–112.3)	7.25 (1.51–34.81)		T1	4.81 (1.73–13.39)	34.30 (5.72–205.7)*
Controls	T0	5.74 (1.13–29.2)	24.47 (3.29–181.9)	Controls	T0	3.43 (1.46–8.05)	11.54 (1.83–72.70)
	T1	37.69 (3.60–294.5)*	119.9 (11.4–1260)*		T1	31.33 (5.50–178.6)*	107.5 (19.16–602.6)*

PCV7, 7-valent pneumococcal conjugate vaccine; PPV, 23-valent pneumococcal polysaccharide vaccine.
MM, multiple myeloma; WM, Waldenstrom's macroglobulinemia; MGUS, monoclonal gammopathy of unknown significance.
T0, baseline (*i. e.*, before vaccination); T1, 4–8 weeks after vaccination.
*$P < 0.05$. Comparison of post vs. pre-vaccination antibody concentrations for each study group, respectively; Wilcoxon signed rank test.
P values for comparisons of vaccine subgroups (PCV7 vs. PPV; pre and post vaccination) are all >0.05 (Mann–Whitney *U* test).

Table 5
Number of subjects with opsonophagocytic (OPA) titers ⩾8 to pneumococcal serotypes 4 and 14, respectively, before and after vaccination with either PCV7 or PPV, and geometric means of OPA titer fold increases.

Study group	Time-point	Serotype 4				Serotype 14			
		OPA titer ⩾8 (%)		OPA fold titer increase		OPA titer ⩾8 (%)		OPA fold titer increase	
		PCV7	PPV	PCV7	PPV	PCV7	PPV	PCV7	PPV
MM	T0	0 (0)	0 (0)			0 (0)	1 (8)		
	T1	3 (25)	2 (17)	2.61	2.25	4 (33)	4 (33)	2.88	3.06
WM	T0	0 (0)	0 (0)			1 (13)	1 (14)		
	T1	2 (25)	2 (29)	2.27	2.15	4 (50)	3 (43)	5.31	3.98
MGUS	T0	0 (0)	0 (0)			0 (0)	2 (22)		
	T1	3 (38)	3 (33)	5.64	2.41	3 (38)	7 (78)	3.63	7.58
Controls	T0	2 (22)	2 (18)			5 (56)	4 (36)		
	T1	5 (56)	6 (55)	6.56	9.14	7 (78)	9 (82)	4.93	9.32

PCV7, 7-valent pneumococcal conjugate vaccine; PPV, 23-valent pneumococcal polysaccharide vaccine.
MM, multiple myeloma; WM, Waldenstrom's macroglobulinemia; MGUS, monoclonal gammopathy of unknown significance.
T0, baseline (*i. e.*, before vaccination); T1, 4–8 weeks after vaccination.
Numbers in parentheses indicate percent responders.
P values for comparisons of vaccine subgroups (PCV7 vs. PPV) regarding OPA titers ⩾8 μg/ml or OPA fold titer increases are all >0.05 (Fisher's exact test and Mann–Whitney *U* test, respectively).

3.5. Serious adverse events

Five unforeseen episodes of hospitalization in four patients were recorded during the follow-up period. Two MM patients were diagnosed with fever of unknown origin. A third MM patient vaccinated with PPV developed pleuritis and multiorgan failure two months after study inclusion, and died. The fourth MM patient succumbed to pneumococcal septicemia two weeks after PCV7 vaccination; the pneumococcal strain was not serotyped and the patient lacked a measurable vaccine response. The fifth case was an MGUS patient who was hospitalized because of myocardial infarction two weeks after vaccination with PPV.

4. Discussion

A recurring problem when comparing studies of pneumococcal vaccine responses is the lack of a consensus definition of immune protection in adults. The World Health Organization has proposed a titer of 0.35 μg/ml as a cut-off level for protection against invasive pneumococcal disease in infants [27]. Although adults generally have higher antibody levels than infants, cut-off IgG titers between 0.15 and 1.0 μg/ml have been used in adult vaccination studies, as well as antibody fold increases [14,24,28]. We chose two cut-off levels, 0.5 and 1.0 μg/ml, to allow for comparisons with previous vaccination studies of adult patients with other hematological malignancies [14,16,23]. The higher cut-off level may be more adequate in our case since an ELISA method without serotype 22F pre-adsorption was used.

As expected, considering their relatively more profound immune dysfunction, the lowest pneumococcal IgG concentrations were found among the MM patients. After vaccination, antibody levels ⩾1.0 μg/ml to all seven serotypes were seen in 38% of the patients, and a statistically significant increase in IgG titers was noted only for one of the seven investigated serotypes. These findings are consistent with previous studies describing low baseline antibody titers and poor pneumococcal vaccine responses among patients with myeloma [10,11]. Low post-immunization antibody levels have further been associated with an increased risk of septicemic episodes in MM patients [10]. Two thirds of our MM patients were on chemotherapy or immunomodulatory medication at the time of the study, and treatment-induced immunosuppression should partly account for the poor vaccine responses in this group. However, we could not demonstrate a statistically significant association between ongoing chemotherapy and OPA titers after vaccination, perhaps because of small patient series. The tendency towards weaker vaccine responses was seen particularly in patients on cyclophosphamide and dexamethasone treatment, a defined risk group for infectious complications [1].

The WM and the MGUS patients displayed lower baseline anti-pneumococcal antibody levels compared to the healthy controls, indicating an increased susceptibility to pneumococcal infection

also among these patients. As we have previously described [12], a stepwise pattern was seen with the lowest antibody titers among MM patients, followed by WM patients, MGUS patients, and healthy controls, respectively. As expected, post-vaccination titers were lower than among the control persons, and 60% of WM patients, 65% of MGUS patients compared to 80% of healthy controls developed IgG antibody levels $\geqslant$1.0 μg/ml to all seven investigated serotypes. MGUS patients are a heterogeneous group. Our patient cohort had a high rate of hypogammaglobulinemia (53%) and high levels of M-protein (median 11 g/L), and had inferior vaccine responses to the healthy controls. Our findings together with recent studies showing increased mortality and morbidity from pneumonia and sepsis among MGUS patients [5,6] suggest MGUS patients should be monitored more closely regarding infectious complications.

The tendency towards lower post-vaccination pneumococcal antibody activity in patients with high M-protein levels and hypogammaglobulinemia compared to those with normal levels of polyclonal serum IgG was expected. Hypogammaglobulinemia has previously been associated with poor vaccine responses among patients with hematological malignancies [1,10,14]. In contrast to other studies [10,14,28] we did not find a relation between age or sex and vaccine response.

The gold standard for evaluation of humoral immunity to pneumococci is measurement of the functionality of the pneumococcal antibodies using a killing-type opsonophagocytosis assay. This is even more essential since the functionality of pneumococcal IgG antibodies seem to wane in the elderly [29,30], which correlates with higher incidence of pneumococcal disease [31]. We selected to measure OPA titers to a poorly immunogenic pneumococcal serotype (4), and a highly immunogenic one (14), both of which are important agents of pneumococcal disease among elderly persons in Sweden [32]. The OPA analyses confirmed the lower IgG antibody titers seen among the patient groups, particularly in MM, both before and after vaccination. The proportions of patients with OPA titers above the chosen reference level of 8, which has been suggested as an equivalent of an IgG level of 0.35 μg/ml, were low; 8% of the MM patients, 27% of the WM patients, 29% of the MGUS patients, and 55% of the healthy controls had OPA titers $\geqslant$8 to both serotypes after vaccination.

Importantly, the very high IgG antibody titers seen in a few MM patients could not be confirmed by the OPA analyses, thus suggesting the presence of cross-reactive, dysfunctional antibodies. Discrepancies between ELISA and OPA results have previously been described in elderly populations [29,30]. Simell et al. showed that significantly higher pneumococcal antibody concentrations were required to achieve the same killing capacity of pneumococci in adults $\geqslant$65 years compared to those <65 years. In contrast, a recent study on allogeneic hematopoietic stem cell transplant patients showed good correlation of post-vaccination ELISA and OPA titers after pneumococcal vaccination [33]. However, these results might not be comparable to ours since the patients had other hematological conditions and were younger (median age 37 years) than in our study.

Conjugate vaccines, in contrast to polysaccharide vaccines, elicit T cell-dependent immune responses and are capable of inducing immunologic memory. It could be anticipated that patients with defective B cells due to malignancies or other B cell disorders would respond better to a T cell-dependent vaccine. However, this study could not confirm the superiority of single-dose vaccination with PCV7 over PPV. Kumar et al. reported slightly better immune responses (defined as a $\geqslant$twofold antibody titer increase and an absolute titer of $\geqslant$0.35 μg/ml) in allogeneic hematopoietic stem cell transplant recipients who had received PCV7 six months after transplantation compared to those who received PPV [34]. The cited vaccination regimen was preceded by vaccination of the healthy stem cell donors with the same vaccine types before transplantation, which makes comparisons difficult. In a study of HIV-infected adults, patients were randomized to receive either PCV7 followed by PPV one month later, or a single dose of PPV. Significantly better antibody responses (defined as a $\geqslant$twofold antibody titer increase and an absolute titer of $\geqslant$1.0 μg/ml) were seen in the prime-boosted study group [24]. Since single dosage was used in our study, it is difficult to draw conclusions regarding the putative efficacy of repeated doses with either of the vaccine types. However, the goal of this study was to evaluate whether a single dose of the conjugate vaccine would be clearly superior to the polysaccharide vaccine, which our results did not support.

The main limitation of this study was the small series of patients. The strengths were that we studied "under-investigated" patient groups, who in addition to high age with concomitant immunosenescence have profoundly depressed humoral immunity. Hence, not only MM patients, but also WM and MGUS patients should be considered as high risk groups for pneumococcal disease. There is little evidence to formulate recommendations for pneumococcal vaccination in these patients to ensure as good protection as possible. A strategy combining conjugate and polysaccharide vaccines is an attractive alternative to enhance vaccine responses and broaden serotype coverage. However, it is of high priority to elucidate the immune mechanisms that underlie the poor vaccine responses to the two conceptually different vaccines to understand how to help these patients the most. A recent study showed that the polysaccharide vaccine gave rise to lower frequencies of pneumococcus-specific switched memory B cells in older adults [35]. Whether this also holds true for immunosuppressed patients with hematological malignancies is presently unknown.

Disclosure

The authors report no conflicts of interest.

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