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**Running head:** IgG2 and pneumococcal vaccination in SLE

**Serum IgG2 levels predict long-term protection following pneumococcal vaccination in systemic lupus erythematosus (SLE)**

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## **ABSTRACT**

Systemic lupus erythematosus (SLE) patients are at risk for pneumococcal infection. Twenty-one consecutive SLE patients (40[25-75] years) received the sequential PCV13/PPSV23 vaccine and factors associated with long-term protection were analyzed. Immune protection, defined by an antigen-specific IgG concentration  $\geq 1.3 \mu\text{g/mL}$  for at least 70% of 7 pneumococcal serotypes was assessed at baseline, 2, 6, 12 and 36 months defining long-term protection. Only 10 patients showed pneumococcal immune protection 36 months after vaccination. Eleven (52.4%) patients had no long-term protection with a seroconversion that never or only transiently occurred. SLE disease features, treatment received and immunological characteristics did not differ between protected and unprotected patients except for the pre-vaccination IgG2 serum levels. Serum IgG2 level  $>2.125\mu\text{g/ml}$  showed a sensitivity of 100% and a specificity of 90.9% for long-term protection. Sequential pneumococcal vaccination conferred poor immune protection in SLE. Baseline IgG2 serum level identified patients able to benefit from pneumococcal vaccination.

**KEYWORDS:** systemic erythematosus lupus; pneumococcal vaccination; immune protection; IgG2

## **INTRODUCTION**

Despite improved management over the last decades, patients with systemic lupus erythematosus (SLE) remain at risk of life-threatening infection [1]. Because pneumococcal infections are both frequent and severe in SLE patients, vaccination against *streptococcus pneumoniae* is now recommended as standard of care using a sequential vaccination, i.e. a PCV13 prime-PPSV23 boost strategy with an interval of at least 8 weeks between the two vaccinations [2-5]. However, the efficacy of such strategy in SLE adult patients remains controversial [6]. Only one published study prospectively addressed the immune efficacy of the PCV13 prime-PPSV23 boost strategy in SLE and showed that less than 60% of patients displayed a sustained immune response against *streptococcus pneumoniae* 52 weeks after the first vaccine injection [7]

In this study, we aimed to measure the immunological response against *streptococcus pneumoniae* 36-month after pneumococcal vaccination using PCV13 in combination with PPSV23 to identify factors associated with long-term immune protection in SLE.

## **METHODS**

### **Study participants**

This prospective monocentric study was conducted in the department of Internal Medicine, Bichat hospital, Paris, France. The inclusion of patients was from April to December 2015. All consecutive SLE patients admitted in our daycare hospital unit with indication for pneumococcal vaccination were enrolled. Patients who had already received pneumococcal vaccination in the past 5 years were excluded. All patients fulfilled at least four of the American College of Rheumatology criteria for SLE [8]. At enrolment, patients were treated with prednisone and/or immunosuppressive (IS) drugs at a stable dose for at least 3 months.

Demographic, SLE-related data -disease activity, lupus nephritis, disease duration, treatment including steroid and immunosuppressive (IS) drugs-, and history of past pneumococcal infection were recorded at inclusion. SLE disease activity was assessed using the Safety of Estrogens in Lupus Erythematosus National Assessment (SELENA)-Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) Score [9]. Lupus nephritis diagnosis was based on International Society of Nephrology/Renal Pathology Society classification [10]. The local ethics committee approved the study (Institutional Review Board IRB 00006477 of HUPNVS, Paris 7 University, AP-HP). All patients provided written informed consent.

### **Vaccination program**

According to the French national recommendations, patients with SLE receiving steroids (> 7.5 mg per day of prednisone for more than 3 months), immunosuppressive drugs or biologics were candidates for pneumococcal vaccination [4]. The recommended protocol was an injection of pneumococcal conjugate vaccine (PCV13) followed 8 weeks later by an injection of pneumococcal polysaccharide vaccine (PPSV23). PCV13 (*Prevenar*®) is a polysaccharid vaccine containing pneumococcal serotypes 1, 3, 4, 5, 6A, 7F, 9V, 14, 18C, 19A, 19F, 23F (2.2 µg) and 6B (4.4 µg), and is individually conjugated to non-toxic diphtheria CRM197 carrier protein (≈32 µg) and adsorbed on aluminium phosphate. PPSV23 (*Pneumo 23*®) is a polysaccharid vaccine countaining pneumococcal serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33F.

### **Assessment of blood B lymphocyte subsets and serum concentrations of immunoglobulins**

Absolute lymphocyte counts were calculated in all SLE patients at baseline from freshly collected blood using the TruCount system (Becton Dickinson, le Pont de la Claix, France)

with CD3-APC, CD45-PerCP-Cy5.5, CD8FITC, and CD4PE mAbs. Eight colors labeling was performed on washed PBMCs with the following mAbs: anti-CD19-PE-Cy7, -CD27-PE, -CD38-V450, -CD10-APC, -CD5-PerCP and -IgD-FITC (Figure S1). Acquisition was performed using a FACSCanto II BD flow cytometer and data were analyzed using FACS Diva (BD Biosciences). Quantification of serum gammaglobulins was performed by electrophoresis. Serum concentrations of IgG, IgA, IgM were assessed by nephelometry. Serum IgG subclass levels were determined by a competitive ELISA method using selected monoclonal antibodies, as previously described [11].

### **Pneumococcal seroconversion following vaccination**

Titers of IgG directed against 7 pneumococcal serotypes (4, 6B, 9V, 14, 18C, 19F, 23F) were measured using a modified enzyme linked immunosorbent (ELISA) [12] at baseline, 2 months, 6, 12 and 36 months after the PCV13 shot in all patients. Sera were pre-absorbed with a solution containing 5µg/ml pneumococcal C-polysaccharide (Statens Serum Institut, Copenhagen, Denmark) and 10µg/ml serotype 22F capsular polysaccharide (American Type Culture Collection). IgG antibody titers were determined by linear regression plots analysis compared to the reference serum (007sp) (National Institute for Biological Standards and Control (NIBSC)). According to previous report [13], immune protection was defined by an IgG-concentration  $\geq 1.3 \mu\text{g/mL}$  for at least 70% of the serotypes following vaccination. Patients had long-term protection (LTP)/no long-term protection (NLTP) when immune protection/no immune protection was observed 36 months after PCV13 shot.

### **Statistical analyses**

Continuous variables are expressed as median [range]. Categorical variables are expressed as numbers and percentages. Data were compared between groups using Fisher test for

dichotomous variables. The Kruskal-Wallis Rank Sum Test was used to compare continuous variables. The Benjamini-Hochberg procedure was used to account for multiple testing. Statistical analysis was performed with R version 3.6.1 and GraphPad Prism 6.0 software. P-values of < 0.05 were considered statistically significant.

## **RESULTS**

### **Characteristics of SLE patients**

Thirty-seven consecutive SLE patients admitted in our daycare hospital unit (Department of Internal Medicine, Bichat Hospital, Paris, France) were asked to participate to a pneumococcal vaccination study. Among them, 8 patients refused to be vaccinated, 7 patients accepted the vaccination but refused to complete the immune response follow up and 1 patient had already been vaccinated against pneumococcal infection. Eventually, 21 SLE patients (40 [25-75] years; 85.7% female) were included.

SLE disease mean duration was 11 [1-32] years. Twelve (57.1%) patients had a past history of lupus nephritis. SLE disease was stable at enrollment, as assessed by a SELENA SLEDAI score of 0 [0-4]. All but 2 (90.5%) patients had received glucocorticoids for 5 [0.5-26] years. Twelve patients (57.1%) were still receiving prednisone at a daily dose of 10 [7-15] mg at study time. Fifteen patients (71.4%) had received immunosuppressive drugs for 4 [1-14] years and 9 (42.9%) patients were still under immunosuppressive drugs at study time. All patients were treated with daily hydroxychloroquine. Three (14.3%) patients had a history of pneumococcal infection prior to vaccination. No patients had received Rituximab during the year before enrollment. No patients had Howell-Jolly bodies on peripheral blood smears.

### **Immune protection following pneumococcal vaccination**

No SLE patient had a protective immune status against pneumococcal infection at baseline. Overall, only 10 SLE patients (47.6%) showed immune protection against pneumococcal infection 36 months after PCV13 shot (long-term protection, LTP). In SLE patients with LTP, immune protection was obtained 2 months after PCV13 shot and maintained overtime in all cases (Figure 1). Conversely, 11 patients had no long-term protection (NTLP) following pneumococcal vaccination, with seroconversion that never (n=6) or only transiently (n=5) occurred. In patients with transient response, immune protection also occurred 2 months after PCV13 shot but was lost 6 (n=2), 12 (n=1) or 36 (n=2) months afterwards. Using corresponding ROC curves (Figure S2), we confirmed [7] that IgG responses measured 2 months after the PCV13 (M2 IgG) shot against serotypes 4 (AUC 0.864 [95% CI: 0.687-1.000], p=0.005), 6B (AUC 0.755 [95% CI: 0.542-0.967]; p=0.05), 9V (AUC 0.900 [95% CI: 0.783-1.000], p=0.0008), 18C (AUC 0.764 [95% CI: 0.545-0.983]; p=0.043), and 19F (AUC 0.845 [95% CI: 0.660-1.000], p=0.006) were predictive of long-term protection. On the other hand, the M2 IgG responses against serotypes 14 (AUC 0.591 [95% CI: 0.332-0.850]; p=0.5), and 23F (AUC 0.691 [95% CI: 0.444-0.938]; p=0.15) were not associated with long-term protection. Of note, invasive pneumococcal infection occurred 3.5 years after PCV13 shot in 1 SLE patient who had no long term immune protection following vaccination. No SLE flare was observed during the first 12 months following pneumococcal vaccination.

### **Baseline IgG2 serum level and immune protection**

To help identifying SLE patients able to reach sustained immune protection after vaccination, we compared the baseline clinical and immunological characteristics between SLE patients with and without long term immune protection. As shown in Table 1, age, SLE features, treatment received, peripheral B-cell subpopulation counts, and serum gammaglobulin levels prior to vaccination were not statistically different between SLE patients, except for a higher

IgG2 levels in LTP (3.30 [2.92, 4.44] g/L) as compared to NLTP (1.45 [1.30, 1.82] g/L,  $p < 0.001$ ). Moreover, the corresponding ROC curve showed that the serum IgG2 level before vaccination was strongly predictive for long-term protection (AUC 0.955 [95% CI: 0.864-1];  $p = 0.0005$ ) (Figure 2). Eventually, a baseline serum IgG2 level of 2.125  $\mu\text{g/mL}$  or more had the best ability to discriminate between LTP and NLTP with a sensitivity of 100% [95% CI: 69-100] and a specificity of 90.9% [95% CI: 61-100] for long-term protection following vaccination

## DISCUSSION

This prospective monocentric study shows that 1- more than 50% of patients with SLE fail to reach immune protection against pneumococcal infection 36 months after vaccination despite a prime-and-boost PCV13/PPSV23 vaccine strategy and 2- baseline IgG2 serum level before vaccination is strongly indicative of long-term protection. Our results also confirm the safety of the vaccine regarding lupus flare-up [6, 14].

IgG2 is known to play a key role in the immune protection against *streptococcus pneumoniae*, [15]. Antibodies directed against pneumococcal polysaccharide antigens are mostly IgG2 [16]. SLE patients may have a high susceptibility for pneumococcal infection because of low IgG2 levels and low binding affinity of IgG2 for bacterial capsules that impair clearance of *Streptococcus pneumoniae* [17, 18]. Interestingly, IgG2 level measured in serum before vaccination was the only factor that differed between protected and unprotected SLE patients. More precisely, all SLE patient with an IgG2 level  $\geq 2.125 \mu\text{g/mL}$  at baseline acquired long term protection, while all but one SLE patient with an IgG2 level  $< 2.125 \mu\text{g/mL}$  failed to reach long term protection. Of note, no SLE patients had defined IgG2 subclass deficiency and all had a preserved serologic immune response against tetanus toxoid (data not shown).

Our study has limitations. First, the number of enrolled patients is limited. Next, we only considered measurements of antibody concentrations with ELISA, and not opsonophagocytic assay (OPA), to assess immune protection. Last, we have no explanation for the variability in IgG2 levels among SLE patients.

Our data confirm the poor immune protection conferred by the sequential PCV13/PPV23 vaccines in SLE patients and highlight the need for innovative vaccination design especially in patients with low IgG2 serum level at baseline.

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## TABLES

**Table 1 Clinical and immunological characteristic of SLE patients**

	LTP (n=10)	NLTP (n=11)	p
Age, years	39.5 [30.7, 41.7]	43 [35.5, 47.5]	ns
Female, n (%)	8 (80)	10 (90.9)	ns
Past history of PI, n (%)	1 (10)	2 (18.2)	ns
Duration of SLE disease, years	13 [6.5, 19]	7 [2, 15]	ns
Lupus nephritis, n (%)	7 (70)	5 (45.5)	ns
Steroid at study time, n (%)	4 (40)	8 (72.7)	ns
Steroid dose at study time, mg/d	9 [7.8, 10.8]	10.00 [7.8, 11]	ns
Cumulative years of steroid	9 [5, 10]	4 [1.25, 6]	ns
IS drugs at study time, n (%)	3 (30)	6 (54.5)	ns
Cumulative years of IS drugs	4.5 [1.2, 9]	1 [0, 3.5]	ns
SELENA-SLEDAI score	1 [0, 2]	0 [0, 2]	ns
Lymphocytes < 1000/mm <sup>3</sup>	2 (20)	3 (27.3)	ns
B cells/mm <sup>3</sup>	124.5 [61, 215.7]	184 [36, 281.5]	ns
Naive B cells/mm <sup>3</sup>	51.5 [34.7, 159.5]	109.5 [36.2, 145]	ns
Transitional B cells/mm <sup>3</sup>	4.8 [1.3, 27.7]	2.1 [0, 11.7]	ns
Unswitched B cells/mm <sup>3</sup>	5.5 [4, 8.7]	13.5 [6.5, 19.7]	ns

Germinal center B cells/mm <sup>3</sup>	0.2 [0, 0.8]	0 [0, 0.2]	ns
Post germinal center B cells/mm <sup>3</sup>	18.4 [16.6, 37.3]	16.2 [13.2, 38.5]	ns
Plasma cells/mm <sup>3</sup>	0.5 [0.2, 1.3]	0.2 [0, 0.4]	ns
Gammaglobulin level < 5 g/L, n (%)	2 (20)	6 (54.5)	ns
IgG, g/L	13.7 [8, 16.2]	9 [4.9, 10.4]	ns
IgG1, g/L	6.4 [4.9, 12.4]	7.1 [4.3, 7.9]	ns
IgG2, g/L	3.3 [2.9, 4.4]	1.3 [1.3-3.4]	<0.001*
IgG3, g/L	0.5 [0.4, 0.7]	0.7 [0.2-1.6]	ns
IgG4, g/L	0.2 [0.1, 0.5]	0.2 [0.2-0.4]	ns
IgA, g/L	2.6 [1.6, 3.2]	2.5 [1.5-3.5]	ns
IgM, g/L	0.89 [0.72, 1.05]	0.8 [0.4-1.4]	ns
Low levels of complement, n (%)	1 (10)	1 (20)	ns

LTP, long-term protection; NLTP, no long term protection; PI, pneumococcal infection; SLE, systemic lupus erythematosus; Lupus nephritis was class III or class IV; IS, immunosuppressive drugs including mycophenolate mofetil, cyclophosphamide, methotrexate and azathioprine; SELENA Safety of Estrogens in Lupus Erythematosus National Assessment, SLEDAI Systemic Lupus Erythematosus Disease Activity Index.

Naive B cells, CD19+CD27-IgD+CD10-CD38-/low cells; transitional B cells, CD19+CD27-IgD+CD10+CD38high; unswitched B cells, CD19+CD27+IgD+ cells; germinal center B cells, CD19+CD27+IgD-CD10+CD38+; post germinal center B cells, CD19+CD27+IgD-CD10-CD38low/+, plasma cells, CD19+CD27+IgD-CD10-CD38high

Significance determined using Kruskal-Wallis Rank Sum Test for continuous variables and Fisher test for categorical variables. ns, no significant (p>0.05). \*Value remains significant after accounting for multiple testing

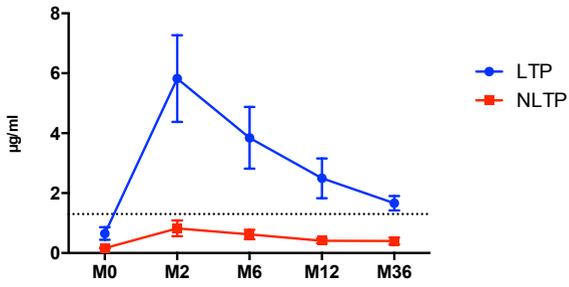
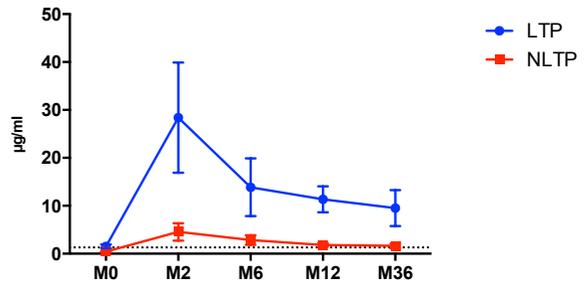
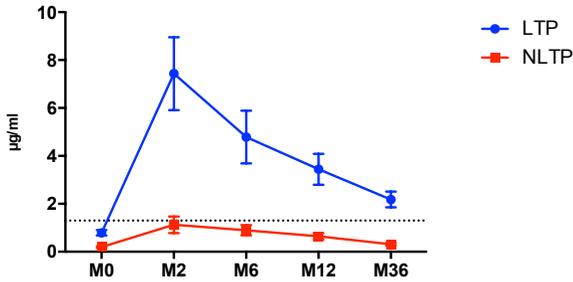
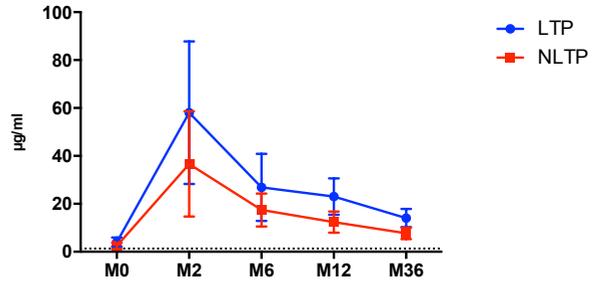
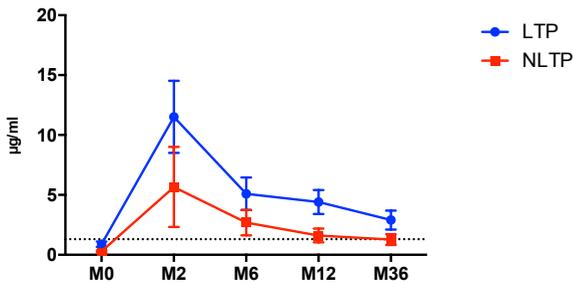
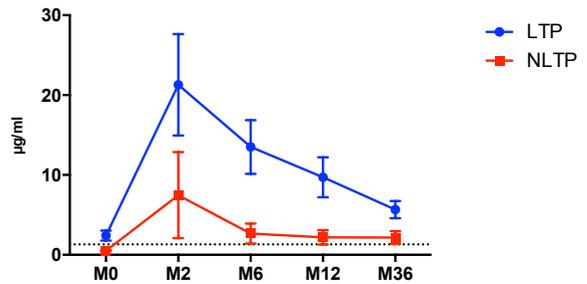
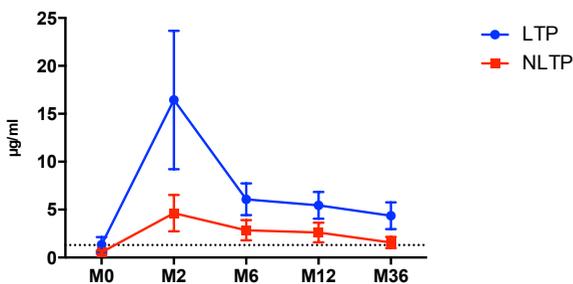
## **FIGURES**

### **Figure 1 Seroconversion following complete pneumococcal vaccination in SLE patients**

Antigen-specific IgG titer ( $\mu\text{g/mL}$ ) directed against pneumococcal serotypes 4, 6B, 9V, 14, 18C, 19F, 23F at baseline, 2, 6, 12 and 36 months after PCV13 shot in SLE patients with (LTP, blue lines) or without (NLTP, red lines) long-term protection. Results are expressed in mean $\pm$ standard error of the mean. Dotted lines represent the immune protection level ( $1.3\mu\text{g/ml}$ ).

### **Figure 2 ROC curves of serum IgG2 levels for prediction of long-term protection in SLE patients**

IgG2 level measured in serum before vaccination is strongly predictive for long-term protection (AUC 0.955 [0.861-1.000],  $p=0.0005$ ). AUC, area under the curve; CI, confident interval

**Serotype 4****Serotype 6B****Serotype 9V****Serotype 14****Serotype 18C****Serotype 19F****Serotype 23F**

# IgG2

