Pneumococcal vaccination in patients with systemic lupus erythematosus: A multicenter placebo-controlled randomized double-blind study

Sophie Grabar, Matthieu Groh, Mathilde Bahuaud, Véronique Le Guern, Nathalie Costedoat-Chalumeau, Alexis Mathian, Thomas Hanslik, Loïc Guillevin, Frédéric Batteux, Odile Launay

Pneumococcal Vaccination in Patients with Systemic Lupus Erythematosus: a Multicenter Placebo-Controlled Randomized Double-Blind Study

Running title: Pneumococcal vaccination in systemic lupus erythematosus

Sophie Grabar[a,b*], Matthieu Groh[c,*§], Mathilde Bahuaud[d], Véronique Le Guern[c], Nathalie Costedoat-Chalumeau[c], Alexis Mathian[f], Thomas Hanslik[g], Loïc Guillevin[c], Frédéric Batteux[d] and Odile Launay [e,h,i], for the VACCILUP study group

[a] Université Paris Descartes, Sorbonne Paris Cité AP-HP, Unité de Biostatistique et Epidémiologie, Groupe Hospitalier Cochin Broca Hôtel-Dieu, Paris, France.

[b] INSERM, UPMC Université Paris 06, Institut Pierre Louis d'épidémiologie et de Santé Publique (IPLESP UMRS 1136), F75013, Paris, France.

[c] Université Paris Descartes, Sorbonne Paris Cité AP-HP, Service de Médecine Interne, Centre de Référence National pour les Maladies Auto-Immunes Rares (Vascularites et Sclérodermie Systémique), Paris, France.

[d] Université Paris Descartes, Sorbonne Paris Cité AP-HP, Département d'Immunologie Biologique, Groupe Hospitalier Cochin Broca Hôtel-Dieu, Paris, France.

[e] Inserm, CIC 1417, Paris, France.

[f] Université Pierre et Marie Curie, Sorbonne Paris Cité AP-HP, Service de Médecine Interne 2, Centre de Référence National pour le Lupus et le Syndrome des Antiphospholipides, institut E3M, Paris, France.

[g] Université Versailles Saint-Quentin-en-Yvelines, APHP, Service de Médecine Interne, Hôpital Ambroise Paré, Boulogne-Billancourt, France.

[h] Université Paris Descartes, Sorbonne Paris Cité AP-HP, Groupe Hospitalier Cochin Broca Hôtel-Dieu, Fédération d'Infectiologie, Paris, France.

[i] Inserm, F-CRIN I-REIVAC.

[*] Dr Grabar and Dr Groh contributed equally to this work.

[§] Present address: Université Pierre et Marie Curie, Sorbonne Paris Cité AP-HP, Department of Internal Medicine, Hôpital St Louis, Paris, France.

Correspondence and requests for reprints to: Pr. Odile Launay, Fédération d'Infectiologie, Hôpital Cochin, 27 rue du Faubourg St Jacques, 75679 Paris cedex 14, France. Phone: +33 (0)1 58 41 28 58 ; Fax : +33 (0)1 58 41 29 10; E-mail : odile.launay@aphp.fr.

Word count: 3463 words, 49 References, 4 Tables, 2 Figures

Funding: Supported by a grant from the "Programme Hospitalier de Recherche Clinique", French Ministry of Health (PHRC 2007-AOM06008).

Disclosure statement: MG, OL: research grant from Pfizer. All other authors have declared no conflicts of interest.

Trial registration: www.clinicaltrials.gov, NCT NCT00611663

Abstract

Background: Invasive pneumococcal disease and respiratory tract infections are both frequent and severe in patients with systemic lupus erythematosus (SLE). This study aimed to compare the immunological efficacy and safety of pneumococcal vaccination with the 23-valent polysaccharide (PPS) vaccine alone to a sequential immunization with the 7-valent pneumococcal conjugate (PnCj) vaccine followed by PPS in patients with SLE and stable diseaase.

Methods: Multicenter randomized placebo-controlled double-blind trial: PPS vaccine alone (placebo-PPS group) or PnCj vaccine followed by PPS vaccine (PnCj-PPS group) 24 weeks later. The primary endpoint was the rate of responders at week 28 to at least 5 of the 7 serotypes (4, 6B, 9V, 14, 18C, 19F and 23F) shared by both PPS and PnCj. Pneumococcal IgG antibodies' opsonophagocytic activity (OPA) were also assessed.

Results: Twenty-five patients in the placebo-PPS group and 17 in the PnCj-PPS group were included in a modified intention-to-treat analysis. The primary endpoint was reached in72% (18/25) in the placebo-PPS and 76% (13/17) in the PnCj-PPS group (p = 0.75). There was no difference in the rates of responders with OPA. At week 52, 13/18 (72%) patients in the placebo-PPS group and 10/13 (77%) patients in the PnCj-PPS group (p=0.77) that met the primary endpoint at week 28 were still responders to ≥ 5/7 serotypes shared by both PPS and PnCj vaccines. Nine SLE flares were reported in 6 patients (4 in the placebo-PPS and 2 in the PnCj-PPS groups respectively, p=0.70).

Conclusion: Sequential administration of PnCj vaccine followed by PPS vaccine is safe and shows short-term immunological efficacy in patients with SLE but was not superior to the PPS vaccine alone.

Keywords: Systemic lupus erythematosus; conjugate pneumococcal vaccine; pneumococcal polysaccharide vaccine, Immunosuppression.

1. Introduction

Despite improvement of survival over the last decades, patients with systemic lupus erythematosus (SLE) have an increased mortality rate as compared with the general population [1,2]. Infections are one of the leading causes of death in this context [2–4]. The excess risk of infections reported during the course of SLE is likely to be multifactorial, with factors (*e.g.* lymphopenia, functional asplenia) inherent to SLE [5] and others (*e.g.* the use of glucocorticoid and/or immunosuppressants) [6,7] acquired during the course of the disease. Both retrospective [8–11] and prospective studies [7] underline that invasive pneumococcal disease and respiratory tract infections are both frequent and severe in the context of SLE. Moreover, pneumonia is the leading cause of avoidable hospitalizations in this setting [12]. Preventing infections is necessary in order to improve both short and long-term prognoses.

Pneumococcal immunization is recommended in immunocompromised hosts [13,14] and in patients with autoimmune inflammatory rheumatic diseases (including SLE), regardless of their level of immunosuppression [15,16]. Previous studies have shown that the 23-valent pneumococcal polysaccharide (PPS) vaccine alone is safe in SLE patients [17–24] but data regarding the short-term immunogenicity of such vaccination are conflicting, and some studies report on a decreased rate of responders among SLE patients as compared to the general population. Moreover, since polysaccharide antigens induce specific antibody production in a T-lymphocyte-independent manner, the 23-valent PPS vaccine is associated

with poor long-term immunogenicity and is unable to prime a booster response in case of subsequent re-exposure [25]. The pneumococcal conjugate (PnCj) vaccine, initially developed for children aged < 2 years (who fail to mount an adequate immune response to the 23-valent vaccine alone), has led to a significant decrease of pneumococcal infections in young infants [26]. In the latter vaccine, polysaccharide antigens are linked to a protein-carrier that stimulates T-helper cells and thus enhances the vaccine's immunogenicity. Previous studies in immunocompromised hosts (*e.g.* HIV infection, Hodgkin's lymphoma, solid organ transplantation) [27–31] have shown that the PnCj vaccine was associated with increased immunogenicity as compared to vaccination with the PPS vaccine alone.

Neither immunization with the PnCj vaccine nor the sequential administrations of both PnCj and PPS vaccines (combined strategy) have been assessed in patients with SLE. The primary objective of this study was, in adult patients with SLE and stable disease, to compare the immunological efficacy and safety of pneumococcal vaccination with the PPS vaccine alone to a vaccination schedule combining PnCJ and PPS vaccines.

2. Patients and methods

2.1. Study population

Patients aged between 18 and 75 years with SLE (defined by the 1997 American College of Rheumatology classification criteria) [32] and stable disease (*i.e.* no modification of the treatment within 2 months before inclusion) were enrolled. Eligible patients had to be treated with at least one of the following drugs: 1) hydroxychloroquine, 2) ≥ 5 mg of daily prednisone or equivalent, 3) systemic glucocorticoids at any dose in combination with at least one immunosuppressant (mycophenolate mofetil, azathioprine or methotrexate). Patients were

excluded if they met one of the following criteria: HIV, HBV or HVC infection; medical history of allergy to any vaccine component; pneumococcal vaccination in the 5 past years; vaccination (any vaccine) in the previous month; intravenous immunoglobulin infusion within three months; splenectomy; bleeding disorders with contraindication to intramuscular injections; active malignancy; cirrhosis; acute infection in the previous month; treatment with rituximab in the previous year. Women of childbearing age without contraception, with a positive urine β-hCG test before vaccination or with a desire of pregnancy within 7 months after inclusion were excluded.

2.2. Study design

The Vaccination in Lupus (VACCILUP, ClinicalTrials.gov NCT00611663) study was a Phase IIb multicenter randomized double-blind placebo-controlled trial comparing two pneumococcal vaccination strategies in patients with SLE. Patients were centrally randomized (1:1) to receive either 1) sequential administration of both the 7-valent PnCj vaccine at baseline followed by the 23-valent PPS vaccine at week 24 (PnCj-PPS group) or 2) vaccination with placebo at baseline and the 23-valent PPS vaccine at week 24 (placebo-PPS group). Randomization was stratified by centers, by the use of immunosuppressants (other than glucocorticoids) and by chronic kidney disease (defined by an estimated GFR <80ml/mn). The "Unité de Recherche Clinique" centrally managed the randomization that was established using a computerized generator that used block size of 4. The study was conducted in 8 rheumatology and internal medicine departments in France. The protocol complied with the Declaration of Helsinki and French law for biomedical research and was approved on the 16th October 2007 by the national Ethic Committee "Comité de Protection

143 des Personnes Ile-de France III" (approval n° 2477). Written informed consent was obtained
144 from each patient.

145 Patients underwent physical examination at inclusion, weeks 4, 24, 28 and 52. Disease
146 activity was assessed using Systemic Lupus Erythematosus Disease Activity Index (SLEDAI)
147 [33] and physician's global disease assessment. Blood samples were collected from all
148 patients at each study visit and tested for routine biochemical, hematological tests (including
149 CD4/CD8 cell counts) and analysis of the immune response induced by the pneumococcal
150 vaccination.

151

152 *2.3. Vaccines*

153 Either Pneumo 23® or Pneumovax 23® (Sanofi Pasteur MSD) were used as 23-valent
154 PPS vaccines targeting serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B,
155 17F, 18C, 19F, 19A, 20, 22F, 23F and 33F of *Streptococcus pneumoniae*. The PnCj vaccine,
156 Prevenar® (Pfizer), is a pneumococcal 7-valent vaccine targeting serotypes 4, 6B, 9V, 14,
157 18C, 19F, 23F of *Streptococcus pneumoniae* in which antigens are conjugated to mutant
158 diphtheria protein CRM_{197}. Vaccines and placebo (serum glucose 5%, 0.5 mL) were
159 administered by intramuscular injections in the deltoid muscle.

160

161 *2.4. Immunogenicity assessments*

162 Immunogenicity measurements were performed in a central laboratory (Cochin
163 hospital) blinded to the trial arm. IgG antibody concentrations for the 7-pneumococcal
164 serotypes shared by both PPS and PnCj vaccines (4, 6B, 9V, 14, 18C, 19F and 23F) were
165 measured at each study visit using a modified enzyme linked immunosorbent
166 [www.vaccine.uab.edu] [34]. Briefly, 96-well plates (Corning, Inc., Corning, NY) were
167 coated with a serotype-specific pneumococcal polysaccharide antigen (American Type

Culture Collection, Manassas, VA) and incubated 5 hours at 37°C. Reference sera (007sp), quality control sera, or patient specimens were pre-absorbed with 5 μg/ml pneumococcal C-polysaccharide (Statens Serum Institut, Copenhagen, Denmark) and 10 μg/ml serotype 22F capsular polysaccharide (American Type Culture Collection) for 30 minutes at room temperature before being serially diluted. After washing plates, serially diluted serum was added and plates were incubated at room temperature for 2 hours. Plates were then washed, and alkaline phosphatase-conjugated goat anti-human immunoglobulin G (IgG; Southern Biotech, Birmingham, AL) was added. After another 2-hour incubation and washing, substrate (p-nitrophenyl phosphate in diethanolamine buffer, pH 9.8) was added to the plates. After a final incubation, the optical density was measured at 405 nm. Anti-pneumococcal antibody levels were determined in each specimen by analysis of linear regression plots compared with the reference serum (007sp).

Opsonophagocytic activities (OPA) were measured at baseline and at week 28 by a multiplexed opsonophagocytic killing assay (MOPA, [www.vaccine.uab.edu] [35]. All serum samples were incubated at 56°C for 30 min before being tested. Sera were serially diluted in round-bottom 96-well plates (Corning Inc., Corning, NY). Frozen aliquots of target pneumococci were thawed, washed twice, diluted to the proper bacterial density, and added to the plates. After 30 min of incubation at room temperature with shaking at 700 rpm, complement and HL60 cells (ATCC) that had been differentiated to phagocytes were added to each well. Plates were incubated in a tissue culture incubator (37°C, 5% CO2) with shaking at 700 rpm. After a 45-min incubation, plates were placed on ice for 20 min. Ten μl of each well were spotted onto four different Todd-Hewitt broth-yeast extract agar plates. After application of an overlay agar containing one of four antibiotics to each agar plate and overnight incubation at 37°C, the number of bacterial colonies in the agar plates was enumerated.

Opsonization titers were defined as interpolated reciprocal serum dilution that kills 50% of the bacteria in the assay. The lowest titer of opsonophagocytic antibody that could be measured by our method was 8, based on the dilution of undiluted serum in the incubation well. Serum specimens not demonstrating a 50% reduction of CFU in the OPA at the lowest serum dilution (1:8) were assigned a titer of 4, enabling statistical analyses of the data sets.

2.5. Safety assessments

Diary cards were provided to patients in order to record injection-site- (pain, erythema, edema, skin nodule) and systemic- (fever, asthenia, headache, arthralgia, myalgia) adverse events (AE) that occurred within 5 days after vaccination. In addition, all AE with a medically attended visit up to 52 weeks after baseline evaluation were recorded at each study visit. Disease activity was assessed at each visit using the SLEDAI score. A flare of SLE was defined as an increase of ≥3 points of the SLEDAI score requiring treatment intensification. An adjudication committee of independent physicians blinded to the trial arm graded the severity of all AE and reviewed all cases of disease flares. SLE flares that occurred after an obvious trigger (*i.e.* treatment non-adherence, tapering of immunosuppression within 2 months prior to the flare, sun exposure) or >12 weeks after the vaccine injection were considered likely not to be related to the vaccination protocol.

2.6. Sample size and statistical analysis

The primary endpoint was the proportion of responders at week 28 to at least 5 of the 7 tested pneumococcal serotypes. The response to a specific serotype was defined as both a 2-fold increase of pneumococcal IgG antibody titers (ELISA) between baseline and week 28 and an antibody titer ≥ 1 µg/mL at week 28. Secondary efficacy endpoints included: serotype-

specific IgG titers at week 28, the rates of patients responding to either 0; 1 or 2; 3 or 4; 5 to 7 serotypes, and the rates of responders to each serotype as assessed by OPA. For the latter, the response to a specific serotype was defined by both at- least a four-fold increase of the opsonization index (OI, defined by the serum dilution killing 50% of the bacterial inoculum) between baseline and week 28, and an OI $\geq$ 8 at week 28. Data were analyzed following a modified intention-to-treat analysis including all patients who received at least the first vaccine or placebo dose. Patients with missing data were considered as non-responders.

Based on the results of the PNEUMOVAC study in HIV-infected patients, we hypothesized that the combined strategy would lead to an increase of 30% of the rate of responders at week 28 (70% vs. 40%) [29]. Taking into account early study discontinuations, with 80% of statistical power and a two-sided alpha risk of 0.05, 53 patients had to be enrolled in both trial arms. Data were analyzed blinded to the trial arm. Patient characteristics are reported as the number and percentage for categorical variables and as the median (IQR) for continuous variables. The percentages of responders in both arms are provided together with their 95% confidence interval (95%CI). Quantitative variables were compared using Student's t-test and categorical variables were compared using the chi-square test. All tests were 2-sided at the level of 0.05. All analyses were performed using SAS version 9.3 (SAS Institute, Inc., Cary, North Carolina, USA).

3. Results

3.1. Study patients

Forty-seven patients were included between May 2008 and November 2011 among which 46 underwent randomization **(Figure 1)**: 27 in the placebo-PPS group and 19 in the PnCj-PPS group. One patient in the placebo-PPS group (blood coagulation disorders) and 2

patients in the PnCj-PPS group (consent withdrawal) did not receive any vaccine. One patient started immunoglobulin infusions after vaccination with placebo but before receiving PPS. Overall, 25 patients in the placebo-PPS group and 17 patients in the PnCj-PPS group were included in a modified intention-to-treat analysis.

The demographic and clinical characteristics of the 2 groups were well balanced and are described in **Table1**. SLE was diagnosed with a median (IQR) of 7.3 [3.7-15.1] years and SLEDAI score was ≥ 4 for 76% of the patients. Overall, 39 (93%) patients were under treatment with antimalarials (hydroxycholoquine or chloroquine) at study entry, 36 (86%) received glucocorticoids (among which 10 (24%) received >10mg of daily prednisone) and 16 (38%) were treated with immunosuppressants (mycophenolate mofetil n=8; azathioprine n=5; methotrexate n=3).

3.2. Immunogenicity according to antibody titers (ELISA)

Four weeks after the first injection (placebo or PnCj vaccine), the rates of responders to at least 5 serotypes shared by both PPS and PnCj vaccines were 0% (0/25) in the placebo-PPS and 35% (6/17) in the PnCj-PPS group ($p=10^{-3}$), while the rates of responders to all 7 serotypes were 0% (0/25) in the placebo-PPS group and 12% (2/17) in the PnCj-PPS group (p=0.08) **(Table 1).**

At week 28 (*i.e.*4 weeks after the PPS injection in both groups), the proportion of responders to at least 5 serotypes shared by both PPS and PnCj vaccines (primary endpoint) was 72% (18/25; 95% CI, 51–88) in the placebo-PPS group and 76% (13/17; 95% CI, 50–93) in the PnCj-PPS group (p=0.75) **(Table 2)**. Patients showing no response to any of the 7-shared serotypes were 16% (4/25) in placebo-PPS group and 18% (3/17) in PnCj-PPS group (p=0.24). Likewise, there was no difference between groups in the rates of responders to the 7

serotypes shared in both vaccines (24% (6/25; 95% CI, 9–45) in the placebo-PPS group and 41% (7/17; 95% CI, 18–67) in PnCj-PPS group (p=0.24)). When using a modified threshold of 0.35µg/ml for antibody titers, the latter definition improved the rates of vaccine response (76.0% in the placebo-PPS versus 76.5% in the PnCj-PPS group, p=1.0) but there was again no difference in between both study groups (Supplemental Tables 1 and 2). Next, there were no differences between the rates of responders in patients (regardless of their vaccine schedule) treated with and without immunosuppressants (75% and 73% respectively, p=1.0), and in those receiving ≤10mg and >10mg of baseline daily prednisone (69% and 90% respectively, p=0.25).

At week 52, 13/18 (72%) patients in the placebo-PPS group and 10/13 (77%) patients in the PnCj-PPS group (p=0.77) that otherwise met the primary endpoint at week 28 were still responders to at least 5 serotypes shared by both PPS and PnCj vaccines. Overall, at week 52, the rates of responders for ≥ 5 serotypes were 52% (13/25) in the placebo-PPS group and 59% (10/17) in the PnCj-PPS group (p=0.66).

3.3. Immunogenicity according to functional antibody titers (OPA)

OPA titers at baseline and at week 28 are reported in **Figure 2**.At week 28, although there was for some serotypes (*e.g.* serotypes 6B, 9V, 18C and 23F) a trend towards better immunogenicity induced by the PnCj-PPS group, the rates of responders to at least 5 serotypes shared by both PnCj and PPS were similar in the two groups: (28% (7/25) in the placebo-PPS group vs. 35% (6/17) in the PnCj-PPS group; p = 0.38).

3.4. Safety

No respiratory tract infection was reported over the study period. At least one AE was reported in 19 patients (76%) of the placebo-PPS group and in 15 patients (88%) of the PnCj-

PPS group (p=0.32). Sixty percent of patients had at least one expected site injection AE, among which pain was the most frequent (**Table 3**). Forty-eight percent of patients had at least one general AE among which headache was the most frequent.

During follow-up, 9 SLE flares were reported in 6 patients (4 in the placebo-PPS group and 2 in the PnCj-PPS group, p=0.70). Such flares occurred within week 24 in 3 and 0 patient(s) in the placebo-PPS and PnCj-PPS groups, respectively (p=0.14) and between weeks 24 and 52 in 2 patients each (p=0.68). A single SLE flare in the placebo-PPS group was considered possibly related to the vaccination protocol. The latter consisted of mild polyarthritis occurring 71 days after vaccination with PPS and resolved after the increase of glucocorticoids. Of note, the rate of patients with SLEDAI scores ≥ 4 and both complement and anti-dsDNA levels remained stable in both groups over the study period (**Table 4**).

4. Discussion

Reducing the burden of infections is a major concern for physicians treating patients with SLE. Means to do so include tapering glucocorticoid [36] and/or immunosuppressant doses, promoting the prescription of antimalarials [8] and timely vaccinations. Current guidelines regarding the prevention of infections of both immunocompromised hosts [13] and patients with autoimmune inflammatory rheumatic diseases [15,16] recommend that pneumococcal immunization be performed. In the present study, sequential administration of the 7-valent PnCj vaccine followed by the 23-valent PPS vaccine was compared to the PPS vaccine alone in patients with SLE and stable disease. Four weeks after the PPS vaccine injection, the rate of responders to at least 5 serotypes of the 7 serotypes shared by both PPS and PnCj vaccines (primary endpoint) was 72% in the placebo-PPS group and 76% in the PnCj-PPS group. After one year, these rates decreased and dropped to 52% in the placebo-

PPS group and 59% in the PnCj group. Hence, unlike previous studies in immunocompromised hosts [29,31] but in line with the study of Tobudic *et al* in 62 renal transplant patients [37] and that of Penaranda in 202 HIV-infected patients [38], we were unable to confirm in our population of SLE patients the potential benefit of a strategy combining the sequential administration of both PnCj and PPS vaccines.

Despite the lack of difference between the rates of responders in the two vaccination groups, it is important to underscore that this trial is the first to assess the safety and efficacy of the PnCj vaccine in patients with SLE. As reported previously in patients with SLE undergoing influenza [39,40] or routine vaccinations [41], pneumococcal vaccination with a conjugated vaccine was safe and did not trigger SLE flares. Next, although there is a time shift between the two vaccination groups, the effects of both PnCj (week 4 of the PnCj-PPS group) and PPS (week 28 of the placebo-PPS group) vaccines alone 4 weeks after immunizations can be compared. Hence, our results suggest that the PnCj vaccine alone was not more immunogenic than the PPS vaccine alone in patients with SLE (**Table 2**). The results with OPA led to the same conclusion (data not shown).

It is unclear why the basal level of antibodies against pneumococcal polysaccharides are found elevated at a level upper than 0,35 □g/ml and even 1□□g/ml in the lupus population (Supplemental Table 2). A report has hypothesized that some anti-dsDNA antibodies might cross-react with bacterial polysaccharides [42] but in our hands we were unable to show any correlation between the levels of antinuclear or anti-phospholipids autoantibodies (data not shown). Moreover, others have already reported such an increase in the basal levels of antibodies against pneumococcal polysaccharides in both SLE [22] and HIV+ [43] pneumococcal vaccine-naïve patients. As pneumococci are commensal bacteria

that can colonize the oropharynx, one might speculate that they might induce subclinical immunization in immunocompromised patients.

Measurement of antibody concentrations with ELISA is the recommended method for market authorization of pneumococcal vaccines. Yet, OPA is considered as the reference method for assessing the protective efficacy of pneumococcal antibodies [44–46]. To our knowledge, the present study is the first to report on OPA titers after pneumococcal vaccination of patients with SLE. Although there was for some serotypes (*i.e.* serotypes 6B, 9V, 18C and 23F) a trend towards better immunogenicity for the PnCj-PPS group (**Figure 2**), there was no overall significant difference at week 28 in the rates of responders for ≥ 5 serotypes assessed by OPA.

Some discrepancies regarding outcomes have been reported in previous studies when both functional antibody levels (OPA) and antibody titers (ELISA) were assessed. In the study of Kumar *et al*, 60 kidney transplant patients received either a single dose of 23-valent PPS vaccine or the 7-valent PnCj vaccine. Although there was a trend towards increased immunogenicity in the PnCj vaccine group with ELISA, no such significant difference was found with OPA [30]. Next, the fact that such differences between ELISA and OPA titers could vary according to the different serotypes of *S. pneumoniae* further brings complexity in the interpretation of data of pneumococcal vaccination trials [28]. Yet, in line with recent studies of allogenic stem cell transplant recipients and HIV-infected patients [43,47], analysis of serotype-specific immune responses in the present study were concordant between both laboratory techniques. Moreover, the rates of responders were lower in both groups with OPA than with ELISA, and neither ELISA nor OPA titers showed a clear superiority of the PnCj-PPS group over the placebo-PPS group.

Our study has some limitations. First, we were not able to enroll the expected number of patients and consequently the study power to detect significant differences between vaccination groups was decreased. Despite growing evidence regarding both the burden of infections and the safety of non-live vaccines in patients with autoimmune inflammatory rheumatic diseases, it is likely that there continues to be an increased risk perception of vaccination by both patients and physicians in this context [48,49]. Next, our results cannot be extrapolated to patients with SLE treated with other biologics (namely B-cell and IFNα-targeted therapies) and/or immunosuppressants (*e.g.* cyclophosphamide or calcineurin inhibitors) that were not included in the present survey. Last, the 7-valent PnCj vaccine was recently replaced in both infants [50], elderly [51] and immunocompromised hosts [13] by a 13-valent PnCj vaccine. Yet, this study is the first to report in patients with SLE on the safety and efficacy of a conjugated vaccine, and to report on vaccines' immunogenicity with the functional OPA.

In conclusion, our results demonstrate the safety and short-term immunological efficacy of both PPS and PnCj vaccines in the context of SLE. Hence, our findings further support the fact that pneumococcal vaccination should be performed in this setting. Yet, priming with PnCj vaccine before PPS vaccine was not superior to PPS vaccine alone in both ELISA and OPA. Last, since more than 40% of patients failed to mount memory immune responses at week 52, our findings underline the need of future studies with new vaccines and/or innovative schedule designs in order to enhance protection of patients with SLE against pneumococcal infections.

Acknowledgements:

The authors thank Philippe Guilpain, Cécile Janssen and Selim Trad for being members of the adverse events' adjudication committee; URC-CIC Paris Descartes Necker/Cochin (Séverine Poignant and Adèle Belleino) and Corinne Desaint (CIC 1417, Paris, France) for trial monitoring and handling, preparation, and submission of all required research ethics and regulatory documents for implementation, monitoring and data management of the study and DEC-AGEPS.

References

[1] Uramoto KM, Michet CJ, Thumboo J, Sunku J, O'Fallon WM, Gabriel SE. Trends in the incidence and mortality of systemic lupus erythematosus, 1950-1992. Arthritis Rheum. 1999;42:46–50.

[2] Bernatsky S, Boivin J-F, Joseph L, Manzi S, Ginzler E, Gladman DD, et al. Mortality in systemic lupus erythematosus. Arthritis Rheum. 2006;54:2550–7.

[3] Cervera R, Khamashta MA, Font J, Sebastiani GD, Gil A, Lavilla P, et al. Morbidity and mortality in systemic lupus erythematosus during a 10-year period: a comparison of early and late manifestations in a cohort of 1,000 patients. Medicine (Baltimore). 2003;82:299–308.

[4] Goldblatt F, Chambers S, Rahman A, Isenberg DA. Serious infections in British patients with systemic lupus erythematosus: hospitalisations and mortality. Lupus. 2009;18:682–9.

[5] Uthman I, Soucy JP, Nicolet V, Senécal JL. Autosplenectomy in systemic lupus erythematosus. J Rheumatol. 1996;23:1806–10.

[6] Noël V, Lortholary O, Casassus P, Cohen P, Généreau T, André MH, et al. Risk factors and prognostic influence of infection in a single cohort of 87 adults with systemic lupus erythematosus. Ann Rheum Dis. 2001;60:1141–4.

[7] Bosch X, Guilabert A, Pallarés L, Cerveral R, Ramos-Casals M, Bové A, et al. Infections in systemic lupus erythematosus: a prospective and controlled study of 110 patients. Lupus. 2006;15:584–9.

[8] Ruiz-Irastorza G, Olivares N, Ruiz-Arruza I, Martinez-Berriotxoa A, Egurbide M-V, Aguirre C. Predictors of major infections in systemic lupus erythematosus. Arthritis Res Ther. 2009;11:R109.

[9] Luijten RKM a. C, Cuppen BVJ, Bijlsma JWJ, Derksen RHWM. Serious infections in systemic lupus erythematosus with a focus on pneumococcal infections. Lupus. 2014;23:1512–6.

[10] Tektonidou MG, Wang Z, Dasgupta A, Ward MM. Burden of Serious Infections in Adults With Systemic Lupus Erythematosus: A National Population-Based Study, 1996-2011. Arthritis Care Res. 2015;67:1078–85.

[11] Feldman CH, Hiraki LT, Winkelmayer WC, Marty FM, Franklin JM, Kim SC, et al. Serious infections among adult Medicaid beneficiaries with systemic lupus erythematosus and lupus nephritis. Arthritis Rheumatol. 2015;67:1577–85.

[12] Ward MM. Avoidable hospitalizations in patients with systemic lupus erythematosus. Arthritis Rheum. 2008;59:162–8.

[13] Rubin LG, Levin MJ, Ljungman P, Davies EG, Avery R, Tomblyn M, et al. 2013 IDSA clinical practice guideline for vaccination of the immunocompromised host. Clin Infect Dis. 2014;58:e44–100.

[14] Centers for Disease Control and Prevention. Advisory Committee on Immunization Practices (ACIP) Recommended Immunization Schedule for Adults Aged 19 Years and Older — United States, 2013. MMWR. 2013;62:9–11.

[15] Assen S van, Agmon-Levin N, Elkayam O, Cervera R, Doran MF, Dougados M, et al.

EULAR recommendations for vaccination in adult patients with autoimmune inflammatory rheumatic diseases. Ann Rheum Dis. 2011;70 :414-22.

[16] Mathian A, Arnaud L, Adoue D, Agard C, Bader-Meunier B, Baudouin V, et al. Prevention of infections in adults and adolescents with systemic lupus erythematosus: Guidelines for the clinical practice based on the literature and expert opinion. Rev Med Interne. 2016;37:307–20.

[17] Klippel JH, Karsh J, Stahl NI, Decker JL, Steinberg AD, Schiffman G. A controlled study of pneumococcal polysaccharide vaccine in systemic lupus erythematosus. Arthritis Rheum. 1979;22:1321–5.

[18] Jarrett MP, Schiffman G, Barland P, Grayzel AI. Impaired response to pneumococcal vaccine in systemic lupus erythematosus. Arthritis Rheum. 1980;23:1287–93.

[19] McDonald E, Jarrett MP, Schiffman G, Grayzel AI. Persistence of pneumococcal antibodies after immunization in patients with systemic lupus erythematosus. J Rheumatol. 1984;11:306–8.

[20] Croft SM, Schiffman G, Snyder E, Herrmann K, James K, Jarrett MP. Specific antibody response after in vivo antigenic stimulation in systemic lupus erythematosus. J Rheumatol. 1984;11:141–6.

[21] Lipnick RN, Karsh J, Stahl NI, Blackwelder WC, Schiffman G, Klippel JH. Pneumococcal immunization in patients with systemic lupus erythematosus treated with immunosuppressives. J Rheumatol. 1985;12:1118–21.

[22] Elkayam O, Paran D, Caspi D, Litinsky I, Yaron M, Charboneau D, et al. Immunogenicity and safety of pneumococcal vaccination in patients with rheumatoid arthritis or systemic lupus erythematosus. Clin Infect Dis. 2002;34:147–53.

[23] Elkayam O, Ablin J, Caspi D. Safety and efficacy of vaccination against streptococcus

453 pneumonia in patients with rheumatic diseases. Autoimmun Rev. 2007;6:312–4.
454 [24] Tarján P, Sipka S, Maródi L, Nemes E, Lakos G, Gyimesi E, et al. No short-term
455 immunological effects of Pneumococcus vaccination in patients with systemic lupus
456 erythematosus. Scand J Rheumatol. 2002;31:211–5.
457 [25] Murdaca G, Orsi A, Spanò F, Puppo F, Durando P, Icardi G, et al. Influenza and
458 pneumococcal vaccinations of patients with systemic lupus erythematosus: current views
459 upon safety and immunogenicity. Autoimmun Rev. 2014;13:75–84.
460 [26] Poehling KA, Talbot TR, Griffin MR, Craig AS, Whitney CG, Zell E, et al. Invasive
461 pneumococcal disease among infants before and after introduction of pneumococcal
462 conjugate vaccine. JAMA. 2006;295:1668–74.
463 [27] Molrine DC, George S, Tarbell N, Mauch P, Diller L, Neuberg D, et al. Antibody
464 responses to polysaccharide and polysaccharide-conjugate vaccines after treatment of
465 Hodgkin disease. Ann Intern Med. 1995;123:828–34.
466 [28] Feikin DR, Elie CM, Goetz MB, Lennox JL, Carlone GM, Romero-Steiner S, et al.
467 Randomized trial of the quantitative and functional antibody responses to a 7-valent
468 pneumococcal conjugate vaccine and/or 23-valent polysaccharide vaccine among HIV-
469 infected adults. Vaccine. 2001;20:545–53.
470 [29] Lesprit P, Pédrono G, Molina J-M, Goujard C, Girard P-M, Sarrazin N, et al.
471 Immunological efficacy of a prime-boost pneumococcal vaccination in HIV-infected adults.
472 AIDS. 2007;21:2425–34.
473 [30] Kumar D, Rotstein C, Miyata G, Arlen D, Humar A. Randomized, double-blind,
474 controlled trial of pneumococcal vaccination in renal transplant recipients. J Infect Dis.
475 2003;187:1639–45.
476 [31] Chan CY, Molrine DC, George S, Tarbell NJ, Mauch P, Diller L, et al. Pneumococcal

conjugate vaccine primes for antibody responses to polysaccharide pneumococcal vaccine after treatment of Hodgkin's disease. J Infect Dis. 1996;173:256–8.

[32] Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1997;40:1725.

[33] Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. Arthritis Rheum. 1992;35:630–40.

[34] Wernette CM, Frasch CE, Madore D, Carlone G, Goldblatt D, Plikaytis B, et al. Enzyme-linked immunosorbent assay for quantitation of human antibodies to pneumococcal polysaccharides. Clin Diagn Lab Immunol. 2003;10:514–9.

[35] Romero-Steiner S, Libutti D, Pais LB, Dykes J, Anderson P, Whitin JC, et al. Standardization of an opsonophagocytic assay for the measurement of functional antibody activity against Streptococcus pneumoniae using differentiated HL-60 cells. Clin Diagn Lab Immunol. 1997;4:415–22.

[36] Tedeschi B, Arnaud L, Hie M, Mathian A, Amoura Z. Successful treatment of combined proliferative and membranous lupus nephritis using a full corticosteroid-free regimen. Ann Rheum Dis. 2014;73:474–5.

[37] Tobudic S, Plunger V, Sunder-Plassmann G, Riegersperger M, Burgmann H. Randomized, single blind, controlled trial to evaluate the prime-boost strategy for pneumococcal vaccination in renal transplant recipients. PloS One. 2012;7:e46133.

[38] Peñaranda M, Payeras A, Cambra A, Mila J, Riera M, Majorcan Pneumococcal Study Group. Conjugate and polysaccharide pneumococcal vaccines do not improve initial response of the polysaccharide vaccine in HIV-infected adults. AIDS. 2010;24:1226–8.

[39] Mathian A, Devilliers H, Krivine A, Costedoat-Chalumeau N, Haroche J, Huong DB-

LT, et al. Factors influencing the efficacy of two injections of a pandemic 2009 influenza A (H1N1) nonadjuvanted vaccine in systemic lupus erythematosus. Arthritis Rheum. 2011;63:3502–11.

[40] Kostianovsky A, Charles P, Alves J-F, Goulet M, Pagnoux C, Le Guern V, et al. Immunogenicity and safety of seasonal and 2009 pandemic A/H1N1 influenza vaccines for patients with autoimmune diseases: a prospective, monocentre trial on 199 patients. Clin Exp Rheumatol. 2012;30:S83–9.

[41] Grimaldi-Bensouda L, Le Guern V, Kone-Paut I, Aubrun E, Fain O, Ruel M, et al. The risk of systemic lupus erythematosus associated with vaccines: an international case-control study. Arthritis Rheumatol. 2014;66:1559–67.

[42] Chowdhry IA, Kowal C, Hardin J, Zhou Z, Diamond B. Autoantibodies that bind glomeruli: cross-reactivity with bacterial antigen. Arthritis Rheum. 2005;52:2403–10.

[43] Bhorat AE, Madhi SA, Laudat F, Sundaraiyer V, Gurtman A, Jansen KU, et al. Immunogenicity and safety of the 13-valent pneumococcal conjugate vaccine in HIV-infected individuals naive to pneumococcal vaccination. AIDS. 2015;29:1345–54.

[44] Jódar L, Butler J, Carlone G, Dagan R, Goldblatt D, Käyhty H, et al. Serological criteria for evaluation and licensure of new pneumococcal conjugate vaccine formulations for use in infants. Vaccine. 2003;21:3265–72.

[45] Romero-Steiner S, Frasch CE, Carlone G, Fleck RA, Goldblatt D, Nahm MH. Use of opsonophagocytosis for serological evaluation of pneumococcal vaccines. Clin Vaccine Immunol. 2006;13:165–9.

[46] Hu BT, Yu X, Jones TR, Kirch C, Harris S, Hildreth SW, et al. Approach to validating an opsonophagocytic assay for Streptococcus pneumoniae. Clin Diagn Lab Immunol. 2005;12:287–95.

525 [47] Cordonnier C, Ljungman P, Juergens C, Maertens J, Selleslag D, Sundaraiyer V, et al.
526 Immunogenicity, safety, and tolerability of 13-valent pneumococcal conjugate vaccine
527 followed by 23-valent pneumococcal polysaccharide vaccine in recipients of allogeneic
528 hematopoietic stem cell transplant aged ≥2 years: an open-label study. Clin Infect Dis.
529 2015;61:313–23.
530 [48] Hanslik T, Wechsler B, Vaillant JN, Audrain L, Prinseau J, Baglin A, et al. A survey
531 of physicians' vaccine risk perception and immunization practices for subjects with
532 immunological diseases. Vaccine. 2000;19:908–15.
533 [49] Lawson EF, Trupin L, Yelin EH, Yazdany J. Reasons for failure to receive
534 pneumococcal and influenza vaccinations among immunosuppressed patients with systemic
535 lupus erythematosus. Semin Arthritis Rheum. 2015;44:666–71.
536 [50] Angoulvant F, Levy C, Grimprel E, Varon E, Lorrot M, Biscardi S, et al. Early Impact
537 of 13-Valent Pneumococcal Conjugate Vaccine on Community-Acquired Pneumonia in
538 Children. Clin Infect Dis. 2014;58:918–24.
539 [51] Bonten MJM, Huijts SM, Bolkenbaas M, Webber C, Patterson S, Gault S, et al.
540 Polysaccharide Conjugate Vaccine against Pneumococcal Pneumonia in Adults. N Engl J
541 Med. 2015;372:1114–25.

542

543 **Figure 1.** Flow chart of patient enrollment

544 **Figure 2.** Rates of responders (assessed by antibodies' opsonophagocytic activity) at week 0
545 and week 28

546