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► To cite this version:

Sophie Grabar, Matthieu Groh, Mathilde Bahuaud, Véronique Le Guern, Nathalie Costedoat-Chalumeau, et al.. Pneumococcal vaccination in patients with systemic lupus erythematosus: A multicenter placebo-controlled randomized double-blind study. *Vaccine*, 2017, 35 (37), pp.4877-4885. 10.1016/j.vaccine.2017.07.094 . hal-03811560

HAL Id: hal-03811560

<https://hal.sorbonne-universite.fr/hal-03811560v1>

Submitted on 12 Oct 2022

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Pneumococcal vaccination in systemic lupus erythematosus

Pneumococcal Vaccination in Patients with Systemic Lupus

Erythematosus: a Multicenter Placebo-Controlled Randomized Double-

Blind Study

Running title: Pneumococcal vaccination in systemic lupus erythematosus

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

38

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

41

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

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45 **Trial registration:** www.clinicaltrials.gov, NCT NCT00611663

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51 Abstract

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

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

63 *Results:* Twenty-five patients in the placebo-PPS group and 17 in the PnCj-PPS group were
64 included in a modified intention-to-treat analysis. The primary endpoint was reached in 72%
65 (18/25) in the placebo-PPS and 76% (13/17) in the PnCj-PPS group ($p = 0.75$). There was no
66 difference in the rates of responders with OPA. At week 52, 13/18 (72%) patients in the
67 placebo-PPS group and 10/13 (77%) patients in the PnCj-PPS group ($p=0.77$) that met the
68 primary endpoint at week 28 were still responders to $\geq 5/7$ serotypes shared by both PPS and
69 PnCj vaccines. Nine SLE flares were reported in 6 patients (4 in the placebo-PPS and 2 in the
70 PnCj-PPS groups respectively, $p=0.70$).

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

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

76

77

78 **1. Introduction**

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

89 Pneumococcal immunization is recommended in immunocompromised hosts [13,14]
90 and in patients with autoimmune inflammatory rheumatic diseases (including SLE),
91 regardless of their level of immunosuppression [15,16]. Previous studies have shown that the
92 23-valent pneumococcal polysaccharide (PPS) vaccine alone is safe in SLE patients [17–24]
93 but data regarding the short-term immunogenicity of such vaccination are conflicting, and
94 some studies report on a decreased rate of responders among SLE patients as compared to the
95 general population. Moreover, since polysaccharide antigens induce specific antibody
96 production in a T-lymphocyte-independent manner, the 23-valent PPS vaccine is associated

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97 with poor long-term immunogenicity and is unable to prime a booster response in case of
98 subsequent re-exposure [25]. The pneumococcal conjugate (PnCj) vaccine, initially developed
99 for children aged < 2 years (who fail to mount an adequate immune response to the 23-valent
100 vaccine alone), has led to a significant decrease of pneumococcal infections in young infants
101 [26]. In the latter vaccine, polysaccharide antigens are linked to a protein-carrier that
102 stimulates T-helper cells and thus enhances the vaccine's immunogenicity. Previous studies in
103 immunocompromised hosts (*e.g.* HIV infection, Hodgkin's lymphoma, solid organ
104 transplantation) [27–31] have shown that the PnCj vaccine was associated with increased
105 immunogenicity as compared to vaccination with the PPS vaccine alone.

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

111

112 2. Patients and methods

113 2.1. Study population

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

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

128

129 *2.2. Study design*

130 The Vaccination in Lupus (VACCILUP, ClinicalTrials.gov NCT00611663) study was
131 a Phase IIb multicenter randomized double-blind placebo-controlled trial comparing two
132 pneumococcal vaccination strategies in patients with SLE. Patients were centrally randomized
133 (1:1) to receive either 1) sequential administration of both the 7-valent PnCj vaccine at
134 baseline followed by the 23-valent PPS vaccine at week 24 (PnCj-PPS group) or
135 2) vaccination with placebo at baseline and the 23-valent PPS vaccine at week 24 (placebo-
136 PPS group). Randomization was stratified by centers, by the use of immunosuppressants
137 (other than glucocorticoids) and by chronic kidney disease (defined by an estimated GFR
138 $<80\text{ml/mn}$). The “Unité de Recherche Clinique” centrally managed the randomization that
139 was established using a computerized generator that used block size of 4. The study was
140 conducted in 8 rheumatology and internal medicine departments in France. The protocol
141 complied with the Declaration of Helsinki and French law for biomedical research and was
142 approved on the 16th October 2007 by the national Ethic Committee “Comité de Protection

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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 CRM₁₉₇. 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

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

180 Opsonophagocytic activities (OPA) were measured at baseline and at week 28 by a
181 multiplexed opsonophagocytic killing assay (MOPA, [www.vaccine.uab.edu] [35]). All serum
182 samples were incubated at 56°C for 30 min before being tested. Sera were serially diluted in
183 round-bottom 96-well plates (Corning Inc., Corning, NY). Frozen aliquots of target
184 pneumococci were thawed, washed twice, diluted to the proper bacterial density, and added to
185 the plates. After 30 min of incubation at room temperature with shaking at 700 rpm,
186 complement and HL60 cells (ATCC) that had been differentiated to phagocytes were added to
187 each well. Plates were incubated in a tissue culture incubator (37°C, 5% CO₂) with shaking at
188 700 rpm. After a 45-min incubation, plates were placed on ice for 20 min. Ten µl of each well
189 were spotted onto four different Todd-Hewitt broth-yeast extract agar plates. After application
190 of an overlay agar containing one of four antibiotics to each agar plate and overnight
191 incubation at 37°C, the number of bacterial colonies in the agar plates was enumerated.

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

197

198 *2.5. Safety assessments*

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

210

211 *2.6. Sample size and statistical analysis*

212 The primary endpoint was the proportion of responders at week 28 to at least 5 of the
213 7 tested pneumococcal serotypes. The response to a specific serotype was defined as both a 2-
214 fold increase of pneumococcal IgG antibody titers (ELISA) between baseline and week 28
215 and an antibody titer ≥ 1 $\mu\text{g/mL}$ at week 28. Secondary efficacy endpoints included: serotype-

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

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

234

235 **3. Results**

236 *3.1. Study patients*

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

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

244 The demographic and clinical characteristics of the 2 groups were well balanced and
245 are described in **Table 1**. SLE was diagnosed with a median (IQR) of 7.3 [3.7-15.1] years and
246 SLEDAI score was ≥ 4 for 76% of the patients. Overall, 39 (93%) patients were under
247 treatment with antimalarials (hydroxychloroquine or chloroquine) at study entry, 36 (86%)
248 received glucocorticoids (among which 10 (24%) received $>10\text{mg}$ of daily prednisone) and 16
249 (38%) were treated with immunosuppressants (mycophenolate mofetil $n=8$; azathioprine $n=5$;
250 methotrexate $n=3$).

251

252 3.2. Immunogenicity according to antibody titers (ELISA)

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

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

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264 serotypes shared in both vaccines (24% (6/25; 95% CI, 9–45) in the placebo-PPS group and
265 41% (7/17; 95% CI, 18–67) in PnCj-PPS group ($p=0.24$)). When using a modified threshold
266 of 0.35 μ g/ml for antibody titers, the latter definition improved the rates of vaccine response
267 (76.0% in the placebo-PPS versus 76.5% in the PnCj-PPS group, $p=1.0$) but there was again
268 no difference in between both study groups (Supplemental Tables 1 and 2). Next, there were
269 no differences between the rates of responders in patients (regardless of their vaccine
270 schedule) treated with and without immunosuppressants (75% and 73% respectively, $p=1.0$),
271 and in those receiving ≤ 10 mg and >10 mg of baseline daily prednisone (69% and 90%
272 respectively, $p=0.25$).

273 At week 52, 13/18 (72%) patients in the placebo-PPS group and 10/13 (77%) patients
274 in the PnCj-PPS group ($p=0.77$) that otherwise met the primary endpoint at week 28 were still
275 responders to at least 5 serotypes shared by both PPS and PnCj vaccines. Overall, at week 52,
276 the rates of responders for ≥ 5 serotypes were 52% (13/25) in the placebo-PPS group and 59%
277 (10/17) in the PnCj-PPS group ($p=0.66$).

278 3.3. Immunogenicity according to functional antibody titers (OPA)

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

284

285 3.4. Safety

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

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

291 During follow-up, 9 SLE flares were reported in 6 patients (4 in the placebo-PPS
292 group and 2 in the PnCj-PPS group, $p=0.70$). Such flares occurred within week 24 in 3 and 0
293 patient(s) in the placebo-PPS and PnCj-PPS groups, respectively ($p=0.14$) and between weeks
294 24 and 52 in 2 patients each ($p=0.68$). A single SLE flare in the placebo-PPS group was
295 considered possibly related to the vaccination protocol. The latter consisted of mild
296 polyarthritis occurring 71 days after vaccination with PPS and resolved after the increase of
297 glucocorticoids. Of note, the rate of patients with SLEDAI scores ≥ 4 and both complement
298 and anti-dsDNA levels remained stable in both groups over the study period (**Table 4**).

299

300 **4. Discussion**

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

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

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

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

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

337 Measurement of antibody concentrations with ELISA is the recommended method for
338 market authorization of pneumococcal vaccines. Yet, OPA is considered as the reference
339 method for assessing the protective efficacy of pneumococcal antibodies [44–46]. To our
340 knowledge, the present study is the first to report on OPA titers after pneumococcal
341 vaccination of patients with SLE. Although there was for some serotypes (*i.e.* serotypes 6B,
342 9V, 18C and 23F) a trend towards better immunogenicity for the PnCj-PPS group (**Figure 2**),
343 there was no overall significant difference at week 28 in the rates of responders for ≥ 5
344 serotypes assessed by OPA.

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

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358 Our study has some limitations. First, we were not able to enroll the expected number
359 of patients and consequently the study power to detect significant differences between
360 vaccination groups was decreased. Despite growing evidence regarding both the burden of
361 infections and the safety of non-live vaccines in patients with autoimmune inflammatory
362 rheumatic diseases, it is likely that there continues to be an increased risk perception of
363 vaccination by both patients and physicians in this context [48,49]. Next, our results cannot be
364 extrapolated to patients with SLE treated with other biologics (namely B-cell and IFN α -
365 targeted therapies) and/or immunosuppressants (*e.g.* cyclophosphamide or calcineurin
366 inhibitors) that were not included in the present survey. Last, the 7-valent PnCj vaccine was
367 recently replaced in both infants [50], elderly [51] and immunocompromised hosts [13] by a
368 13-valent PnCj vaccine. Yet, this study is the first to report in patients with SLE on the safety
369 and efficacy of a conjugated vaccine, and to report on vaccines' immunogenicity with the
370 functional OPA.

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

379

380 **Acknowledgements:**

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

387

388 **References**

- 389 [1] Uramoto KM, Michet CJ, Thumboo J, Sunku J, O'Fallon WM, Gabriel SE. Trends in
390 the incidence and mortality of systemic lupus erythematosus, 1950-1992. *Arthritis Rheum.*
391 1999;42:46–50.
- 392 [2] Bernatsky S, Boivin J-F, Joseph L, Manzi S, Ginzler E, Gladman DD, et al. Mortality
393 in systemic lupus erythematosus. *Arthritis Rheum.* 2006;54:2550–7.
- 394 [3] Cervera R, Khamashta MA, Font J, Sebastiani GD, Gil A, Lavilla P, et al. Morbidity
395 and mortality in systemic lupus erythematosus during a 10-year period: a comparison of early
396 and late manifestations in a cohort of 1,000 patients. *Medicine (Baltimore).* 2003;82:299–308.
- 397 [4] Goldblatt F, Chambers S, Rahman A, Isenberg DA. Serious infections in British
398 patients with systemic lupus erythematosus: hospitalisations and mortality. *Lupus.*
399 2009;18:682–9.
- 400 [5] Uthman I, Soucy JP, Nicolet V, Sénécal JL. Autosplenectomy in systemic lupus
401 erythematosus. *J Rheumatol.* 1996;23:1806–10.
- 402 [6] Noël V, Lortholary O, Casassus P, Cohen P, Généreau T, André MH, et al. Risk
403 factors and prognostic influence of infection in a single cohort of 87 adults with systemic
404 lupus erythematosus. *Ann Rheum Dis.* 2001;60:1141–4.

Pneumococcal vaccination in systemic lupus erythematosus

- 405 [7] Bosch X, Guilabert A, Pallarés L, Cerveral R, Ramos-Casals M, Bové A, et al.
406 Infections in systemic lupus erythematosus: a prospective and controlled study of 110
407 patients. *Lupus*. 2006;15:584–9.
- 408 [8] Ruiz-Irastorza G, Olivares N, Ruiz-Arruza I, Martinez-Berriotxoa A, Egurbide M-V,
409 Aguirre C. Predictors of major infections in systemic lupus erythematosus. *Arthritis Res Ther*.
410 2009;11:R109.
- 411 [9] Luijten RKM a. C, Cuppen BVJ, Bijlsma JWJ, Derksen RHWM. Serious infections in
412 systemic lupus erythematosus with a focus on pneumococcal infections. *Lupus*.
413 2014;23:1512–6.
- 414 [10] Tektonidou MG, Wang Z, Dasgupta A, Ward MM. Burden of Serious Infections in
415 Adults With Systemic Lupus Erythematosus: A National Population-Based Study, 1996-2011.
416 *Arthritis Care Res*. 2015;67:1078–85.
- 417 [11] Feldman CH, Hiraki LT, Winkelmayr WC, Marty FM, Franklin JM, Kim SC, et al.
418 Serious infections among adult Medicaid beneficiaries with systemic lupus erythematosus and
419 lupus nephritis. *Arthritis Rheumatol*. 2015;67:1577–85.
- 420 [12] Ward MM. Avoidable hospitalizations in patients with systemic lupus erythematosus.
421 *Arthritis Rheum*. 2008;59:162–8.
- 422 [13] Rubin LG, Levin MJ, Ljungman P, Davies EG, Avery R, Tomblyn M, et al. 2013
423 IDSA clinical practice guideline for vaccination of the immunocompromised host. *Clin Infect*
424 *Dis*. 2014;58:e44–100.
- 425 [14] Centers for Disease Control and Prevention. Advisory Committee on Immunization
426 Practices (ACIP) Recommended Immunization Schedule for Adults Aged 19 Years and Older
427 — United States, 2013. *MMWR*. 2013;62:9–11.
- 428 [15] Assen S van, Agmon-Levin N, Elkayam O, Cervera R, Doran MF, Dougados M, et al.

Pneumococcal vaccination in systemic lupus erythematosus

- 429 EULAR recommendations for vaccination in adult patients with autoimmune inflammatory
430 rheumatic diseases. *Ann Rheum Dis.* 2011;70 :414-22.
- 431 [16] Mathian A, Arnaud L, Adoue D, Agard C, Bader-Meunier B, Baudouin V, et al.
432 Prevention of infections in adults and adolescents with systemic lupus erythematosus:
433 Guidelines for the clinical practice based on the literature and expert opinion. *Rev Med*
434 *Interne.* 2016;37:307–20.
- 435 [17] Klippel JH, Karsh J, Stahl NI, Decker JL, Steinberg AD, Schiffman G. A controlled
436 study of pneumococcal polysaccharide vaccine in systemic lupus erythematosus. *Arthritis*
437 *Rheum.* 1979;22:1321–5.
- 438 [18] Jarrett MP, Schiffman G, Barland P, Grayzel AI. Impaired response to pneumococcal
439 vaccine in systemic lupus erythematosus. *Arthritis Rheum.* 1980;23:1287–93.
- 440 [19] McDonald E, Jarrett MP, Schiffman G, Grayzel AI. Persistence of pneumococcal
441 antibodies after immunization in patients with systemic lupus erythematosus. *J Rheumatol.*
442 1984;11:306–8.
- 443 [20] Croft SM, Schiffman G, Snyder E, Herrmann K, James K, Jarrett MP. Specific
444 antibody response after in vivo antigenic stimulation in systemic lupus erythematosus. *J*
445 *Rheumatol.* 1984;11:141–6.
- 446 [21] Lipnick RN, Karsh J, Stahl NI, Blackwelder WC, Schiffman G, Klippel JH.
447 Pneumococcal immunization in patients with systemic lupus erythematosus treated with
448 immunosuppressives. *J Rheumatol.* 1985;12:1118–21.
- 449 [22] Elkayam O, Paran D, Caspi D, Litinsky I, Yaron M, Charboneau D, et al.
450 Immunogenicity and safety of pneumococcal vaccination in patients with rheumatoid arthritis
451 or systemic lupus erythematosus. *Clin Infect Dis.* 2002;34:147–53.
- 452 [23] Elkayam O, Ablin J, Caspi D. Safety and efficacy of vaccination against streptococcus

Pneumococcal vaccination in systemic lupus erythematosus

- 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, Neuberger 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, Pédrone 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

Pneumococcal vaccination in systemic lupus erythematosus

- 477 conjugate vaccine primes for antibody responses to polysaccharide pneumococcal vaccine
478 after treatment of Hodgkin's disease. *J Infect Dis.* 1996;173:256–8.
- 479 [32] Hochberg MC. Updating the American College of Rheumatology revised criteria for
480 the classification of systemic lupus erythematosus. *Arthritis Rheum.* 1997;40:1725.
- 481 [33] Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of the
482 SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in
483 SLE. *Arthritis Rheum.* 1992;35:630–40.
- 484 [34] Wernette CM, Frasch CE, Madore D, Carlone G, Goldblatt D, Plikaytis B, et al.
485 Enzyme-linked immunosorbent assay for quantitation of human antibodies to pneumococcal
486 polysaccharides. *Clin Diagn Lab Immunol.* 2003;10:514–9.
- 487 [35] Romero-Steiner S, Libutti D, Pais LB, Dykes J, Anderson P, Whitin JC, et al.
488 Standardization of an opsonophagocytic assay for the measurement of functional antibody
489 activity against *Streptococcus pneumoniae* using differentiated HL-60 cells. *Clin Diagn Lab*
490 *Immunol.* 1997;4:415–22.
- 491 [36] Tedeschi B, Arnaud L, Hie M, Mathian A, Amoura Z. Successful treatment of
492 combined proliferative and membranous lupus nephritis using a full corticosteroid-free
493 regimen. *Ann Rheum Dis.* 2014;73:474–5.
- 494 [37] Tobudic S, Plunger V, Sunder-Plassmann G, Riegersperger M, Burgmann H.
495 Randomized, single blind, controlled trial to evaluate the prime-boost strategy for
496 pneumococcal vaccination in renal transplant recipients. *PloS One.* 2012;7:e46133.
- 497 [38] Peñaranda M, Payeras A, Cambra A, Mila J, Riera M, Majorcan Pneumococcal Study
498 Group. Conjugate and polysaccharide pneumococcal vaccines do not improve initial response
499 of the polysaccharide vaccine in HIV-infected adults. *AIDS.* 2010;24:1226–8.
- 500 [39] Mathian A, Devilliers H, Krivine A, Costedoat-Chalumeau N, Haroche J, Huong DB-

Pneumococcal vaccination in systemic lupus erythematosus

- 501 LT, et al. Factors influencing the efficacy of two injections of a pandemic 2009 influenza A
502 (H1N1) nonadjuvanted vaccine in systemic lupus erythematosus. *Arthritis Rheum.*
503 2011;63:3502–11.
- 504 [40] Kostianovsky A, Charles P, Alves J-F, Goulet M, Pagnoux C, Le Guern V, et al.
505 Immunogenicity and safety of seasonal and 2009 pandemic A/H1N1 influenza vaccines for
506 patients with autoimmune diseases: a prospective, monocentre trial on 199 patients. *Clin Exp*
507 *Rheumatol.* 2012;30:S83–9.
- 508 [41] Grimaldi-Bensouda L, Le Guern V, Kone-Paut I, Aubrun E, Fain O, Ruel M, et al.
509 The risk of systemic lupus erythematosus associated with vaccines: an international case-
510 control study. *Arthritis Rheumatol.* 2014;66:1559–67.
- 511 [42] Chowdhry IA, Kowal C, Hardin J, Zhou Z, Diamond B. Autoantibodies that bind
512 glomeruli: cross-reactivity with bacterial antigen. *Arthritis Rheum.* 2005;52:2403–10.
- 513 [43] Bhorat AE, Madhi SA, Laudat F, Sundaraiyer V, Gurtman A, Jansen KU, et al.
514 Immunogenicity and safety of the 13-valent pneumococcal conjugate vaccine in HIV-infected
515 individuals naive to pneumococcal vaccination. *AIDS.* 2015;29:1345–54.
- 516 [44] Jódar L, Butler J, Carlone G, Dagan R, Goldblatt D, Käyhty H, et al. Serological
517 criteria for evaluation and licensure of new pneumococcal conjugate vaccine formulations for
518 use in infants. *Vaccine.* 2003;21:3265–72.
- 519 [45] Romero-Steiner S, Frasch CE, Carlone G, Fleck RA, Goldblatt D, Nahm MH. Use of
520 opsonophagocytosis for serological evaluation of pneumococcal vaccines. *Clin Vaccine*
521 *Immunol.* 2006;13:165–9.
- 522 [46] Hu BT, Yu X, Jones TR, Kirch C, Harris S, Hildreth SW, et al. Approach to validating
523 an opsonophagocytic assay for *Streptococcus pneumoniae*. *Clin Diagn Lab Immunol.*
524 2005;12:287–95.

Pneumococcal vaccination in systemic lupus erythematosus

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