

(Online supplementary text)

METHODS

ELISAs for serotype-specific IgG

Sera were collected immediately before and 4 to 6 weeks after vaccination, and stored at -30°C until tested. For the measurement of IgG specific to serotypes 6B and 23F, ELISAs were performed according to the World Health Organization (WHO) standard procedure that uses the international reference serum, 89SF-3 (graciously supplied by Dr. Carl E. Frasch). To improve the ELISA specificity, a pneumococcal cell wall polysaccharide (C-PS) and pneumococcal 22F polysaccharide pre-absorption step was performed on the patients' sera. The reference serum was pre-absorbed with only C-PS.^{1,2} Detailed protocols are available at [www.vaccine.uab.edu/ELISAProtocol\(89SF\).pdf](http://www.vaccine.uab.edu/ELISAProtocol(89SF).pdf).

Multiplexed OPAs

To measure functional antibody activity against pneumococcus, we performed multiplexed OPAs for pneumococcal serotypes 6B and 23F, using differentiated HL-60 cells and antibiotic-resistant target bacteria strains, at the Research Institute for Microbial Disease, Osaka University, as previously described.³ The quality control serum was prepared from pooled sera of adults immunized with PPV23 and included in each assay. An opsonization index (OI) was defined as the serum dilution that led to 50% killing of target bacteria. Opsotiter3, an excel-based data processing program, was used to convert colony counts to OIs, according to the WHO protocol, available at www.vaccine.uab.edu/UAB-MOPA.pdf.

Statistical analysis

In univariate analyses for categorical variables, differences between treatment groups were analyzed using the chi-square test or Fisher's exact probability test. Continuous variables were assessed using

the Mann-Whitney *U*-test for nonparametric data comparisons between the two treatment groups. Parametric and nonparametric data comparisons between the four treatment groups were performed using either ANOVA (analysis of variance) with post-hoc Turkey's HSD (honesty significant difference) test or the Kruskal-Wallis test with post-hoc Scheffe test. Differences in IgG concentrations or OIs between pre-vaccination and post-vaccination were compared using the paired-sample *t* test. Multivariate logistic regression analysis was used to assess the relationship between positive antibody response to each pneumococcal serotype and a set of predictor variables including age, RA duration, positive status of anti-cyclic citrullinated peptide antibodies, positive rheumatoid factor status, current MTX use, current prednisolone use, ongoing TCZ use, lymphocyte counts ($< 1000/\mu\text{l}$), and serum IgG ($< 1000 \text{ mg/dl}$). A backward stepwise selection procedure was used to select significant independent variables. Pearson's correlation coefficient was used to assess the strength of the relationship between IgG concentrations and OIs. For all tests, probability values (*p* values) < 0.05 were considered to indicate statistical significance. All calculations were performed using Excel Statistical Analysis 2008 (SSRI Co., Ltd., Tokyo, Japan) or PASW Statistics version 20 (SPSS Japan Inc., Tokyo, Japan).

REFERENCES

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2. Wernette CM, Frasch CE, Madore D, *et al.* Enzyme-linked immunosorbent assay for quantitation of human antibodies to pneumococcal polysaccharides. *Clin Diagn Lab Immunol* 2003;10(4):514-9.
3. Burton RL, Nahm MH. Development and validation of a fourfold multiplexed opsonization assay (MOPA4) for pneumococcal antibodies. *Clin Vaccine Immunol* 2006;13(9):1004-9.