**Effect of Age, Sex and Smoking Habits on Pneumococcal Antibodies in an Elderly Population**

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Sankilampi U (National Public Health Institute, Department in Oulu, PO Box 310, FIN-90101 Oulu, Finland), Isoaho R, Bloigu A, Kivelä S-L and Leinonen M. Effect of age, sex and smoking habits on pneumococcal antibodies in an elderly population. *International Journal of Epidemiology* 1997; 26: 420–427.

**Background.** Pneumococcal infections are a common cause of morbidity and mortality among elderly people. Protection against pneumococcal infections is mediated by serotype-specific antibodies to capsular polysaccharides. To obtain an estimate of anti-pneumococcal immunity, prevalence and levels of pneumococcal antibodies were studied in an unvaccinated elderly population.

**Methods.** IgG antibodies to pneumococcal serotypes 3, 6A, and 8 and to cell wall polysaccharide (C-PS, a common antigen to all pneumococci) were measured by enzyme immuno-assay in 480 subjects aged 64–97 years (206 men, 274 women) who were a random sample (41%) of elderly inhabitants in a semirural community in Finland.

**Results.** An average of 10% of the elderly lacked antibodies to serotypes 3, 6A, and 8, and 62% of the elderly had them in low titres only. Anti-C-PS antibodies were found in 99% of the elderly, and in significantly higher titres than anti-capsular antibodies. Antibody titres to C-PS and to type 6A decreased with age. Elderly women had significantly lower antibody levels than men. Among the men, current smokers had higher antibody titres than non-smokers; in the women, this analysis was not possible because of infrequent history of smoking. The effect of smoking on antibody titres was reversible after cessation of smoking.

**Conclusions.** A considerable proportion of the elderly lacked protective antibodies to commonly infecting pneumococcal serotypes 3, 6A, and 8. Smoking increased the prevalence and levels of pneumococcal antibodies probably as a consequence of numerous respiratory infections. These observations emphasize the importance of administration of the pneumococcal vaccine among the elderly.

**Keywords:** *Streptococcus pneumoniae*, antibodies, elderly, ageing, sex, smoking

*Streptococcus pneumoniae* is the most common bacterial agent causing community-acquired pneumonia in the elderly, and a frequent cause of bacteraemia and meningitis as well. The incidence of pneumonia increases with age, and the highest morbidity and case fatality rates are seen in elderly men.1 A 23-valent capsular polysaccharide vaccine has been available for prophylaxis of pneumococcal infections since 1983, but its utilization is still suboptimal. In the US, less than 30% of the elderly have received pneumococcal vaccination despite general recommendations to vaccinate all those aged 65 years or over, and inclusion of the vaccination in the free-of-charge Medicare programme.2,3 In Finland and other European countries, pneumococcal vaccine is only recommended to small risk groups, such as the splenectomized, if at all.

In all, 90 pneumococcal serotypes have been identified on the basis of the antigenic differences of their capsular polysaccharides.4 The 20 most common types cause more than 80% of invasive pneumococcal infections in adults.5,6 All pneumococci, independent of the serotype, share a common cell-wall polysaccharide (C-PS).7 Both pneumococcal infections and nasopharyngeal carriage stimulate the synthesis of serotype-specific anti-capsular antibodies, as well as antibodies to the C-PS.8–10 The anti-capsular antibodies provide protection against invasive pneumococcal infections in a serotype-specific manner, whereas antibodies to C-PS appear less protective, and may not be protective at all.11–13 Most studies in which anti-capsular antibodies have been measured have utilized methods with impure antigens containing both the capsular polysaccharide and C-PS, and thus not distinguishing between antibodies to these two antigens.14–17 Only recently, enzyme immuno-assays (EIA) have been developed that allow...
the specific measurement of anti-C-PS antibodies on one hand (using purified C-PS as antigen) and of anti-
capsular antibodies on the other (by blocking of anti-
C-PS antibodies before the assay). These methods
have so far been mainly applied to studies in children,
and the information on the prevalence and concentra-
tions of these antibodies among the elderly is very
limited.17–19

Antibodies to the pneumococcal capsular polysac-
charides, induced either naturally or by vaccination, play
a central role in the specific defence against pneumococ-
cal infections. Thus, an estimate of anti-pneumococcal
immunity in the elderly may be obtained by measuring
the prevalence and concentrations of anti-capsular
antibodies to pneumococcal serotypes frequently caus-
ing invasive infections. In addition to ageing, several
chronic conditions, as well as smoking, increase the
risk of pneumonia and pneumococcal infections.20–22
Nevertheless, the existing recommendations on the use
of pneumococcal vaccine include old age and some
chronic illnesses, but not smoking, as indications for
vaccination,2 and no data are available on the levels of
pneumococcal antibodies in smokers.

The aim of this seroepidemiological study was to
investigate the prevalence and levels of pneumococcal
antibodies, and explore their associations with age, sex,
and smoking habits, in an elderly population in a semi-
rural community. Antibodies were measured to C-PS
and to capsular polysaccharides of serotypes 3, 6A, and 8,
commonly found in invasive pneumococcal infections.
The study was part of a research project on the epidemi-
ology of respiratory diseases in the elderly (the Lieto
study).23

MATERIALS AND METHODS

Study Population and Sampling

The present study was conducted in Lieto, a semirural
Characteristics of the survey population and protocol
have been described in detail elsewhere.23 All the inhabi-
tants of Lieto born in or before 1926 (N = 1360) were
invited in a random order to the Health Centre for ex-
aminations by one of the study investigators (RI) and a
trained nurse. Seventy-seven subjects died before they
were examined. Of the remaining 1283 subjects, 1196
(488 men and 708 women, 93% of those eligible) partic-
ipated. A venous blood sample was drawn for antibody
assays from 1174 subjects (92% of those eligible). None
of the participants had ever received a pneumococcal
vaccination. Current and previous smoking habits were
recorded by means of the British Medical Research
Council (MRC) questionnaire.24 An ex-smoker was
defined as anyone who had smoked as much as one
cigarette per day (or equal amount of other tobacco) for
as long as a year, and who at the time of the examina-
tion had not smoked for ≥6 months. The cumulative
exposure to tobacco was measured in pack-years; a
pack-year was defined as smoking one pack of cigare-
ettes (or equal amount of other tobacco) daily for a year.

For the present seroepidemiological study, we
included the first 467 subjects who attended the survey.
Because the participants of the Lieto study were invited
in a random order, the first 467 subjects were a random
sample of the whole survey population constituting
40% of those surveyed. This sample included six men
in the oldest age group of ≥85 years. To increase their
number, all the 13 men aged ≥85 years from the remain-
ing part of the survey population were also included.
The final study population of the present study thus
consisted of 480 subjects (mean age 73.8 years, range
64–97 years; 206 men, 274 women) (41% of the survey
population). In all 296 people (133 men, 163 women)
belonged to the age group 64–74 years, 138 (54 men,
84 women) to the age group 75–84 years, and 46 (19 men,
27 women) to the age group ≥85 years. The mean ages
of men and women within age groups were similar.
Because history of smoking was rare among the women
(seven women were current smokers, 13 were ex-
smokers, and 254 women had never smoked), only men
were included when the effects of smoking habits on
the antibody levels were analysed.

The sera were stored at –20°C until assayed.

Antibody Determination

IgG antibodies to pneumococcal cell wall polysac-
charide (C-PS) and to the capsular polysaccharides of sero-
types 3, 6A, and 8 were measured by EIA.10,19

Enzyme immuno-assay for anti-C-PS antibodies. Cell
wall polysaccharide was isolated from a pneumococcal
mutant strain with a capsule consisting of C-PS
(C-mutant CSR, SCS-2, clone 1, received from Dr J
Henrichsen, Statens Seruminstitut, Copenhagen).25

Microtitre plates (Maxisorp, Nunc, Roskilde, Denmark)
were coated with C-PS in phosphate buffered saline
(PBS) (5 μg/ml) by incubating for 5 h at 37°C and then
overnight at 4°C. After three washes with PBS contain-
ing 0.05% Tween, the plates were postcoated with 10%
fetal bovine serum (FBS; GIBCO-BRL, Glasgow, UK)
in PBS (FBS-PBS) for 1 h at 37°C.

Sera were diluted 1:100, 1:1000, and 1:10 000 in
FBS-PBS, and the dilutions were incubated as duplic-
cates for 2 h at 37°C. Then the plates were washed as
previously. Alkaline phosphatase-conjugated goat anti-
human IgG (Sigma Chemical Co., St Louis, MO, USA)
diluted in FBS-PBS was incubated for 2 h at 37°C. After washing with two additional H2O washes in the end, p-nitrophenyl phosphate substrate (Sigma) was incubated in the wells for 30 minutes at 37°C. Enzyme reaction was stopped with 2 N NaOH. Optical densities were read at 405 nm by an EIA reader (Labsystems Multiscan MCC/340, Labsystems, Helsinki). The serum end point titre was interpolated from the intersection of the OD versus serum dilution curve at 0.3 OD units. Corrected end point titres were obtained by comparing the end point titre of each serum sample to that of an in-house reference serum on the same plate, which was calibrated to its mean of 35 consecutive measurements. The results are expressed as corrected end point titres.

**Enzyme immuno-assay for anti-capsular antibodies.** Before measuring type-specific anti-capsular antibodies in the sera, the anti-C-PS antibodies were neutralized in a pre-incubation step. The sera were diluted 1:100 in FBS-PBS containing 20 μg/ml of C-PS to block the anti-C-PS antibodies. After incubation of 30 minutes at room temperature, further dilutions (1:1000 and 1:10 000) were made in FBS-PBS.

Microtitre plates were coated with 10 μg/ml of pneumococcal capsular polysaccharides 3, 6A, and 8 (American Type Culture Collection, Rockville, MD, USA). The EIA with the absorbed sera was then performed as described above for anti-C-PS antibodies.

The sensitivity of the assay was around the titre of 100 for antibodies to C-PS and to the capsular polysaccharides tested. Serum antibody titres <100 were considered as negative, titres 100–999 as low, and titres ≥1000 as high.

**Statistical Analysis**

Statistical analyses were performed with SPSS (SPSS, Chicago, USA). The antibody titres were converted into their natural logarithms for calculation of the geometric mean titres (GMT). The analyses of variance and covariance (with age as a covariate), the χ² test, the Student’s t test, and confidence interval (CI) analysis were used for testing the statistical significance as indicated.

**RESULTS**

*Prevalence of Antibodies*

Anti-C-PS antibodies were found in the sera of all but three (99%) of the 480 elderly. Most of them (91%) had high titres (≥1000) (Table 1). In men, the percentage of subjects with a high titre was similar in all age groups, whereas in women, the percentage decreased significantly from 93% in the age group 64–74 years to 74% in the age group >85 years (P < 0.01, χ² test).

Anti-capsular antibodies to serotypes 3, 6A, and 8 were detected in the sera of 89%, 86%, and 93% of the elderly, but in high titres (≥1000) in only 21%, 38%, and 24% of them, respectively. High titres to the three capsular polysaccharides were significantly less frequent than high titres to C-PS (P < 0.01 for each comparison, 99% CI analysis). Elderly men had high antibody titres to the three serotypes more frequently than women (P < 0.01 for types 6A and 8; P = 0.13 for type 3, χ² test).

An average of 10% of the subjects lacked antibodies to serotypes 3, 6A, and 8 compared to 1% in respect of C-PS (P < 0.01 for each comparison, 99% CI analysis). Ageing did not have an effect on this percentage in men.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>% of subjects with antibody titre &lt;100</th>
<th>% of subjects with antibody titre 100–999</th>
<th>% of subjects with antibody titre ≥1000</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (men/women)</td>
<td>Total (men/women)</td>
<td>Total (men/women)</td>
</tr>
<tr>
<td>C-PS</td>
<td>1 (0/1)a</td>
<td>8 (5/11)b</td>
<td>91 (95/88)b</td>
</tr>
<tr>
<td>Type 3</td>
<td>11 (8/12)</td>
<td>68 (68/69)</td>
<td>21 (24/19)</td>
</tr>
<tr>
<td>Type 6A</td>
<td>14 (9/18)b</td>
<td>48 (46/50)</td>
<td>38 (45/32)c</td>
</tr>
<tr>
<td>Type 8</td>
<td>7 (5/8)</td>
<td>69 (63/74)c</td>
<td>24 (32/18)c</td>
</tr>
</tbody>
</table>

NOTE. Antibody levels are expressed as corrected end point titres.

a C-PS versus serotypes 3, 6A, and 8; P < 0.01, 99% confidence interval analysis.

b Men versus women, P < 0.05, χ² test.

c Men versus women, P < 0.01, χ² test.
or women (data not shown). Women lacked antibodies to the capsular polysaccharides of all three serotypes more often than men, but the difference was statistically significant only for type 6A (9% of men versus 18% of women, \( P < 0.05, \chi^2 \) test) (Table 1).

**Mean antibody titres by age and sex.** Geometric mean titres (GMT) of antibodies to C-PS and to the three pneumococcal capsular polysaccharides in elderly men and women are shown in Figure 1. The GMT of antibodies to C-PS significantly decreased with increasing age in both sexes \( (P < 0.001, \) analysis of variance). The decrease of anti-C-PS antibodies was most evident in the oldest age group (>85 years). Antibodies to serotype 6A decreased, like the anti-C-PS antibodies, with age \( (P < 0.01) \), whereas GMT of antibodies to types 3 and 8 were not significantly associated with age in either sex.

Elderly women had lower GMT than elderly men: this was true for antibodies to C-PS and to the three serotypes and for every age group (difference was statistically significant for types 6A and 8 and for C-PS, \( P < 0.01, \) analysis of variance).

**Antibody titres by smoking habits in elderly men.** Thirty-one men were current smokers, 107 were ex-smokers and 68 had never smoked (non-smokers). The mean number of pack-years in the ex-smokers was 20 (SD 18) and in current smokers 44 (SD 23). Current smokers were younger (mean age 69.1 years, SD 7.7 years) than ex-smokers (mean 73.5 years, SD 7.5 years) or non-smokers (mean 75.0 years, SD 7.7 years). Current smokers had the highest antibody titres to C-PS and to the three serotypes studied, and the ex-smokers had higher titres than the non-smokers (Table 2). This trend remained after adjustment for age so that the difference between the three smoking status groups was statistically significant for anti-C-PS and anti-6A-antibodies \( (P < 0.001, \) analysis of covariance with age as a covariate).

In current smokers and ex-smokers, no correlation was found between antibody titres and the duration of smoking, either between the daily exposure (number of cigarettes per day) or cumulative exposure (pack-years) and antibody titres. The ex-smokers were classified into three groups according to the smoking-free period: those who had stopped smoking more than 30 years ago \( (N = 46) \), those who had stopped 16–30 years ago \( (N = 38) \), and those who had stopped 1–15 years ago \( (N = 21) \); data of two ex-smokers were not available.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Non-smokers ((N = 68))</th>
<th>Ex-smokers ((N = 107))</th>
<th>Current smokers ((N = 31))</th>
<th>(P)-values, ANOVA/ covariate (age)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GMT (a) (95% CI)</td>
<td>Age-a. (b) GMT</td>
<td>GMT (95% CI)</td>
<td>Age-a. GMT</td>
</tr>
<tr>
<td>C-PS</td>
<td>3790 (3041–4722)</td>
<td>4022</td>
<td>5378 (4403–6568)</td>
<td>5491</td>
</tr>
<tr>
<td>Type 3</td>
<td>483 (365–639)</td>
<td>486</td>
<td>493 (407–590)</td>
<td>491</td>
</tr>
<tr>
<td>Type 6A</td>
<td>550 (392–765)</td>
<td>568</td>
<td>721 (561–934)</td>
<td>734</td>
</tr>
<tr>
<td>Type 8</td>
<td>478 (372–614)</td>
<td>495</td>
<td>665 (550–796)</td>
<td>674</td>
</tr>
</tbody>
</table>

**NOTE.** Antibodies are expressed as corrected end point titres. An ex-smoker was defined as anyone who had smoked regularly; i.e. as much as one cigarette per day (or equal amount of other tobacco) for as long as a year and who had not smoked for \( \geq 6 \) months.

\(a\) Geometric mean titre.  
\(b\) Age-adjusted.
The average smoking-free periods in these groups were 43 (SD 7), 22 (SD 4), and 7 (SD 4) years, respectively. Figure 2 demonstrates the individual antibody titres to C-PS and serotype 6A of non-smokers, ex-smokers (shown separately in the three smoking-free groups), and current smokers. The longer time had passed from the cessation of smoking, the lower GMT the ex-smoker men had to C-PS and to type 6A. Ex-smokers who had stopped smoking 1–15 or 16–30 years ago, had significantly higher GMT to C-PS than non-smokers (P < 0.005 and < 0.05, respectively, Student’s t test). Ex-smokers with 1–15 smoking-free years also had significantly higher GMT to serotype 6A than non-smokers (P < 0.05). The women were not included in the analysis, because most of them (93%) had never smoked. Instead, we compared the antibody levels of the 68 non-smoker men with those of the 254 non-smoker women (mean age 74.5 years, SD 7.4 years). Unlike in the comparison of all elderly men and women, the GMT to types 3, 6A and 8 and to C-PS did not differ statistically significantly between the non-smoker men and women (P > 0.27 for all antigens, Student’s t test). However, the trend of elderly men having higher antibody titres than women also remained among the non-smokers (data not shown).

DISCUSSION
The results of this population-based study show that practically all the elderly had high levels of serum antibodies to pneumococcal C-PS. Most of them had detectable antibodies to pneumococcal capsular polysaccharides of three common serotypes (3, 6A, and 8), but in low levels only. Geometric means of antibody titres to C-PS and to type 6A, but not to types 3 and 8, decreased with increasing age. Elderly women had lower antibody levels than men to all antigens studied and in all age groups. Smoking seemed to significantly increase the pneumococcal antibody levels.

Serogroups/types 3, 6, and 8 were chosen as the capsular antigens for the assays because of two reasons. Firstly, they are a frequent cause of severe disease together responsible for 30% of invasive pneumococcal infections among the elderly in Finland. Secondly, at least in children, they differ on their immunogenicity profiles, group 6 being a poor immunogen and types 3 and 8 good ones. Antibodies to serotypes 6A and 6B of group 6 are highly cross-reactive, and in the present work we used type 6A polysaccharide as an antigen to represent group 6. The method of choice for measuring serum antibodies to the pneumococcal capsular polysaccharides nowadays is EIA with blocking
of anti-C-PS antibodies, as applied in the present study. A standard serum with known concentrations (µg/ml) of antibodies to certain pneumococcal serotypes has recently become available for antibody measurements; unfortunately serotypes 3, 6A, and 8 do not belong to these serotypes, and thus the results of the present study are expressed as corrected endpoint titres. There is no standard serum available for the anti-C-PS antibodies, either.

Antibodies to C-PS were found in the sera of 99% of the elderly, and in 91% of them in high titres (≥1000). The GMT were the highest in the youngest age group, 64–74 years, and decreased with ageing both in men and women. Antibodies to C-PS are common in sera of almost all adults, and this finding is now extended to the elderly. Cell wall polysaccharide is a common cell wall component of all the pneumococci, and also found in another Streptococcus-species, Streptococcus mitior (which is sometimes present in the pharyngeal normal flora, but seldom causes disease). Recently, cross-reactions have also been described between pneumococcal C-PS and structures of Haemophilus influenzae. Antibodies to C-PS in the population thus reflect the cumulative exposure to pneumococci and other bacteria containing cross-reactive structures.

The majority of the elderly had antibodies to serotypes 3, 6A, and 8, although in significantly lower levels than anti-C-PS antibodies. Unlike the anti-C-PS antibodies, serotype-specific anti-capsular antibodies have been shown to confer protection from invasive pneumococcal disease, but the protective concentrations are not known. In all, 11% of the elderly lacked antibodies to type 3, which is the most common serotype among pneumococcal isolates from the elderly in Finland, causing alone 16% of their invasive pneumococcal infections. The highest percentage (14%) of the elderly lacked antibodies to serogroup 6, which is a common cause of invasive paediatric infections, but less frequent in invasive infections of the elderly than e.g. type 3. Antibodies to type 6A decreased with increasing age whereas antibodies to types 3 and 8 remained constant (Figure 1). Almost one-quarter of those aged ≥85 years lacked antibodies to serotype 6A.

In the present study the elderly men had higher antibody concentrations to the three capsular polysaccharides and to C-PS than the women of the same age. This observation confirms earlier observations of Roghmann et al. and ours showing that elderly women have lower antibody concentrations than men both before and after pneumococcal vaccination. The reason for this difference between the sexes is not known, but seemed to be partially due to their different smoking habits: when the comparison between men and women was repeated among the non-smokers only, no statistically significant difference in the antibody titres was observed. Smoking is rare among elderly Finnish women; also in the present population only 3% of the women were current smokers compared to 15% of the men. In this study, we found higher pneumococcal antibody titres in elderly men who were current smokers than in those who had stopped smoking or had never smoked (Table 2). Smoking increases the risk of respiratory infections, including pneumococcal pneumonia. Elevated anti-capsular polysaccharide antibody titres in current smokers may reflect more numerous pneumococcal infections and/or a higher carrier rate of pneumococci than in ex- or non-smokers. Elevated anti-C-PS antibody levels in current smokers may be less specific than the anti-capsular antibody levels: in addition to pneumococci, colonization and/or infection by other respiratory bacteria possessing cross-reactive structures may increase their levels. The observation of ex-smokers having higher pneumococcal antibody levels than non-smokers even after 16–30 smoking-free years is intriguing, and might reflect either longevity of the antibodies or slow recovery of the pulmonary defence mechanisms.

Effects of smoking on systemic immunity have been examined in both humans and experimental animals. In smokers, the number of polymorphonuclear leukocytes, as well as the total number of T and B cells in the peripheral blood, are increased. In contrast, serum concentrations of IgA, IgM, and IgG have been 10–20% lower than in non-smokers. The immune response to inhaled antigens has been found lower, and the antibody levels following vaccination with the influenza vaccine have declined more rapidly in smokers than non-smokers. Also the mucosal immunity of smokers has been shown to be altered with significantly lower salivary IgA and IgG concentrations than in non-smokers. The immune response to inhaled antigens has been found lower, and the antibody levels following vaccination with the influenza vaccine have declined more rapidly in smokers than non-smokers. An explanation for the impaired immune response in smokers may lie in changes in the T cell function.

We observed that elderly smokers had higher IgG antibody levels to pneumococcal polysaccharide antigens than non-smokers, which is contrary to the above data showing decreased total IgG concentrations and impaired immune responses in smokers. Pneumococcal capsular polysaccharides, as well as other bacterial polysaccharides, are prototypes of T cell independent antigens, i.e. antigens which directly stimulate antibody production by B cells without the help of T cells. A suppressed antibody response to T cell dependent antigens has been observed in mice chronically exposed to tobacco smoke, whereas the response to T cell independent antigens remained normal. A similar analysis in humans has, to our knowledge, not been reported.
before, and our observations suggest that the same may be true for humans. The increased risk for pneumococcal infections in smokers may then not be dependent on a poor serum antibody response to pneumococcal antigens, but rather on other factors, e.g. the altered function of alveolar macrophages and impaired mucociliary clearance observed in smokers.\textsuperscript{30,35} Salivary antibodies to pneumococcal antigens, especially IgA, may also play a role in the defence against pneumococcal colonization and infection,\textsuperscript{36} and their concentrations in smokers remain to be analysed. So far, there are no studies on the immunogenicity of the 23-valent pneumococcal vaccine among smokers, either.

Even though anti-C-PS antibodies were found in sera of nearly all the elderly, an average of 10% of the elderly did not have any capsular antibodies to the three pneumococcal capsular types, 3, 6A, and 8. All those with detectable antibody levels to types 3, 6A, and 8 may not be protected against invasive pneumococcal infection, either. The majority of the elderly had antibodies to these three serotypes in low titres only, all of which may not be high enough for providing protection. In this context it is important to know that the serum antibody concentrations could be increased with vaccination, and that the 23-valent pneumococcal vaccine has shown good immunogenicity also in the elderly.\textsuperscript{19} Together with the rapid emergence of drug-resistant pneumococci all over the world,\textsuperscript{37,38} these observations emphasize the importance of administration of the pneumococcal vaccine among the elderly.

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REFERENCES


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