Seroprevalence of antibodies against serogroup C meningococci in England in the post-vaccination era

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Running title: Meningococcal serogroup C seroprevalence in England
ABSTRACT

The UK introduced meningococcal serogroup C conjugate (MCC) vaccines in 1999, resulting in substantial declines in serogroup C disease and carriage. Here, we measured the age-specific prevalence of serum bactericidal antibodies (SBA) to Neisseria meningitidis serogroup C and immunoglobulin G (IgG) concentrations to serogroups A, C, W-135 and Y in 2,673 serum samples collected in England between 2000 and 2004. We compared the seroprevalence of SBA titers $\geq 8$ in the post-vaccination era, with an earlier, pre-vaccination study conducted using the same methods. We found that the percentages of individuals with protective SBA titers were higher in 2000-2004 in all of the age groups targeted for MCC vaccination. In the post-vaccine era the prevalence of protective titers was high (75%) in children who had recently been offered routine immunization, but this fell to 36% more than 18 months after scheduled immunization. In the cohorts targeted in the catch-up campaign, the percentage achieving SBA titers $\geq 8$ was higher in children offered vaccine at age 5-17 years compared to age 1-4 years. Geometric mean concentration (GMC) IgG for serogroup C followed a similar pattern, corresponding to the age at and time since scheduled MCC vaccination. Serogroup-specific IgG GMCs for W-135 and Y were low and showed little variation by age. Serogroup A IgG GMCs were higher, possibly reflecting exposure to cross-reacting antigens. Although the incidence of serogroup C disease remains low due to persisting herd effects, population antibody levels to serogroup C meningococci should be monitored, so that potentially susceptible age-groups can be identified should herd immunity wane.
INTRODUCTION

The UK was the first country to introduce meningococcal serogroup C conjugate (MCC) vaccination in 1999, incorporating MCC vaccines into the routine infant immunization schedule at 2, 3, 4 months of age, and implementing an extensive catch-up campaign targeting children and young adults up to the age of 18 years (later extended to 24 years) (19). Since then, the incidence of serogroup C disease has declined markedly, as a result of high short-term vaccine effectiveness (29) and a reduction in serogroup C carriage leading to herd immunity (15,16,24). The MCC vaccines were licensed on the basis of immunogenicity and safety trials, in the absence of a large phase III efficacy trial. This was possible because well-defined immunologic correlates of protection for serogroup C disease existed. It is widely accepted that serum bactericidal antibody (SBA) titers $\geq 4$ (with human complement) and $\geq 8$ (with baby rabbit complement) are indicative of protection from meningococcal disease (1-3,7). As well as providing the basis for pre-licensure evaluation of vaccines (19), such surrogates of protection are also useful for post-licensure surveillance.

Serologic surveillance has been used in the UK to inform vaccine policy for several diseases, for example, measles (6) and Haemophilus influenzae type b (Hib) (31). Continued meningococcal disease surveillance is obviously crucial to monitor the long-term impact of the MCC vaccine programme, but serologic surveillance can be used to complement this. In England, we observed that the effectiveness of MCC waned rapidly in children immunized with a 2, 3, 4 month schedule (29). In response to this, the vaccine schedule was changed in September 2006, so that now children in the UK are offered MCC vaccines at 3, 4 and 12 months (5). The later dose at 12 months (given in combination with Hib) is expected to improve and extend direct protection in those children born after September 2005. The number of cases of serogroup C cases remains low in all ages, including the cohort of children.
immunized according to a 2, 3, 4 month schedule, probably as a result of low transmission
and continued herd immunity. It is uncertain how long these herd effects may last.

Seroprevalence studies can be used to improve our understanding of population immunity and
to identify groups who are likely to be susceptible to serogroup C meningococcal infection
should herd protection wane.

We previously reported the seroprevalence of SBA to serogroup C meningococci in the pre-
vaccine era. (28). This earlier serosurvey used the same laboratory methods to test residual
sera from the same populations and as the present study, and thus provides a baseline measure
of ‘natural’ immunity, against which we can compare population immunity in the presence of
MCC vaccination. Here, we examined the age-specific seroprevalence profile for bactericidal
serogroup C antibodies using sera collected in the post-vaccination era between 2000 and
2004. In addition, we also measured IgG antibodies against Neisseria meningitidis serogroups
A, C, W-135 and Y.

MATERIALS AND METHODS

Serum samples

Sera were obtained from the Health Protection Agency (HPA) Seroepidemiology Unit. This
collection is described fully elsewhere (22), but briefly, participating laboratories submit
residual sera from routine diagnostic testing to the collection. Samples are anonymized, a
unique identity number is assigned and details regarding the age and sex of the donor, year of
collection and the participating laboratory are collated on a database. The reason why the
blood sample was taken is not documented, but immunocompromized individuals are
excluded.
Sera collected between 2000 and 2004 (up to five years after the introduction of MCC vaccination) were selected for analysis. For each year samples were selected randomly within age-group and region strata. Sample sizes targets were about 40 per year (2001-2004) for age 0, 1, 2, 3, 4, 5, 6, 7, 8/9, 10/11, 12/13 14/15, 16/17 and 18/19 years. This sample size was chosen to enable reasonable precision of proportions with SBA titers ≥8 within strata of interest (e.g. combining 2 age groups gives n=80 per age group, which would enable a proportion of 0.5 to be estimated with 95% CI 0.39-0.61). In some ages there were insufficient samples available to meet these targets. Since the MCC catch-up campaign was rolled out during 2000, we did not select any sera from under 20 year olds in this year, so all sera in 0-19 year olds is from 2001-2004. For adults (age 20+ years) samples were tested from those available in the year 2000. A total of 2673 samples were chosen across the age range, from individuals aged between 0 and 93 years. Some results were not obtained due to insufficient volumes; the numbers of sera by age group for which results were obtained are shown in table 1.

Serum bactericidal antibody assay

Sera were tested using standardized complement-mediated SBA against O-acetylated serogroup C strain C11 (C:16:P1.7-1,1) as previously described (17). The complement source was pooled 3-4 week old baby rabbit serum (Pel-Freeze Biologicals WI, USA). SBA titers were expressed as the reciprocal of the final serum dilution giving ≥ 50% killing at 60 minutes. SBA titers <4 were assigned a value of 2 for computational purposes. Results were obtained for 2391 samples.

Serum IgG concentrations
Serogroup-specific IgG concentrations were determined in serum by use of a tetraplex IgG bead assay for serogroups A, C, W-135 and Y as previously described (14). The calibration factors of the standard CDC1992 serum for *N. meningitidis*-specific IgG used were 91.8 µg/mL for serogroup A, 24.1 µg/mL for serogroup C (10), 25.23 µg/mL for serogroup W-135 and 28.92 µg/mL for serogroup Y (12). The lower limits of detection were 0.08, 0.06, 0.065 and 0.075 µg/mL for serogroups A, C, W-135 and Y, respectively. For any values recorded below this threshold, a value equal to half the lower limit of detection was assigned for computational purposes. Results were obtained for 2658 samples.

**Statistical Analysis**

Results from the serologic analyzes were linked to information on the age and sex of the donor. The age of the serum donor and the year of sample collection were used to infer the age and time at which MCC vaccination is likely to have been offered, according the routine and catch-up immunization schedule (19). Since all samples were anonymized, it was not possible to collect information on immunization status. The percentage of individuals, by age, with SBA titers ≥8 was calculated, together with 95% confidence intervals (95% CI). For individuals aged less than 20 years, the age-specific prevalence of SBA titers ≥8 against serogroup C meningococci was compared according to the age at vaccination/schedule that the group was eligible to have received and the time since vaccination was offered (within 2 years, or after 2 years or more). A two-sample, two-sided z-test was used to assess the equality of proportions. The geometric mean concentrations (GMC) of serogroup-specific IgG were calculated, by age, with corresponding 95% CI. The correlation between serogroup C IgG and SBA titers in individuals was assessed using the Spearman’s rho statistic. Data were analyzed using Stata version 10.0.
Comparison with pre-vaccine study

The age-specific prevalence of SBA to serogroup C between 2000 – 2004 was compared with the results of a previously published seroprevalence study from the pre-vaccine era (28). This earlier study used the same source of sera (the HPA seroepidemiology collection, described above) and the same standardized laboratory methods, performed by the same laboratory.

Ethical approval

The HPA Seroepidemiology Unit has ethical approval from the Joint University College London/ University College London Hospitals Committees on the Ethics of Human Research (REC reference number 05/Q0505/U5) for serological surveillance of the National Immunization Programme for England and Wales.

RESULTS

The cross-sectional age-specific prevalence of SBA titers $\geq 8$ in the post-vaccine era is shown in figure 1, and compared with the seroprevalence profile before the introduction of MCC vaccines. In those aged under 20 years (the main age range targeted in the MCC campaign), the percentage with SBA titers $\geq 8$ is higher in the post-vaccine compared to pre-vaccine era (p<0.001 for all age-groups). In sera collected from infants less than one year old, 63.1% (95% CI 51.9 to 73.4%) achieved protective titers in 2001-2004, with a higher proportion (p<0.001) with SBA titers $\geq 8$ in those aged 6-11 months compared to <6 months (figure 1). In children aged 1-4 years, the percentage with protective titers is lower than observed in infants at 35.4% (95% CI 31.6% to 39.4%, p<0.001). In older children, the percentage with protective titers increases, and in 10-14 and 15-19 year olds, the prevalence of SBA titers $\geq 8$ is around 67%. In sera collected from those aged 25 to 64 years prevalence is similar to that in the pre-vaccine era (as shown by overlapping confidence intervals in figure 1). In those aged
65 years or older, however, the prevalence of protective titers is significantly lower post-
vaccine introduction (p<0.001).

The percentage of children with protective titers stratified by both age at and time since
scheduled vaccination is shown in figure 2. In children scheduled for immunization in infancy
at 2, 3, 4 months, the percentage with SBA ≥8 declines from 74.6% within 18 months to
36.2% 18 months or more after vaccination was scheduled (p<0.001). In children offered
vaccine at older ages, no significant differences are observed according to time since
immunization. The proportion of children achieving (and maintaining) SBA titers ≥8 is higher
in those offered vaccine at older ages (5 and above) compared to those offered vaccine at 1-4
years of age (as shown by non-overlapping confidence intervals in figure 2).

The serogroup and age-specific IgG GMCs are shown in figure 3. For serogroup C, the
distribution of GMCs by age are broadly similar to that for SBA titers, and is consistent with
coverage of and time since MCC immunization. There was evidence of correlation between
IgG GMC and SBA titers for serogroup C, with Spearmans rho of 0.6 (p<0.001). The IgG
GMC for individuals with an SBA titer >=8 was 4.2 (95% CI 3.8 to 4.7) ug/ml, compared to
0.61 (95% CI 0.57 to 0.65) ug/ml in individuals with an SBA titer <8.

Serogroup-specific IgG GMCs for serogroups W-135 and Y were low, and showed little
variation by age. For anti-serogroup A, we observed a sharp increase in IgG GMC from age 0
to 1 year, followed by a plateau, with a further increase in older ages. The serogroup-specific
IgG GMC for serogroup A was much higher than for serogroups W-135 and Y and also
significantly higher than serogroup C for all ages apart from <1 year olds and 12-19 years
olds.
DISCUSSION

We have described the age-specific prevalence of SBA and IgG antibodies to serogroup C meningococci in the English population following the introduction of MCC vaccines. As anticipated, a higher proportion of individuals achieved SBA titers $\geq 8$ in all age groups targeted for MCC immunization compared to the pre-vaccine seroprevalence profile. We observed variations in the proportions achieving putative protective titers according to vaccine schedule offered, in particular the age at which vaccination was scheduled. In the children targeted for routine immunization at 2, 3, 4 months, we observed that the prevalence of putative protective SBA titers declined with time since vaccination. Previous studies have shown that antibody persistence is poor after MCC immunization in infancy (at 2, 3, 4 months) and in children immunized at a young age (less than 2 years) in the catch-up campaign (4,26). This appears to be reflected in our results, where only 32% of children aged 1-4 years had SBA titers $\geq 8$. For those targeted in the catch-up campaign, a higher percentage of children had SBA titers $\geq 8$ if they were offered vaccine at 5 –17 years of age, compared to those targeted at 1-4 years of age. This is consistent with estimates of vaccine effectiveness in England, which suggested that protection was higher, and more persistent, in those immunized above the age of 3 years, compared to 1-2 years old (29), and with higher coverage in the school-based cohorts compared to pre-school children aged 2-5 years (32).

Furthermore, studies of antibody persistence after vaccination have shown greater persistence when vaccine was administered at older ages (26,27).

The results of this cross-sectional study are compatible with those observed in studies that have followed cohorts of vaccinated children over time. Snape and colleagues reported that of 94 children who were immunized with a single dose of MCC around 2 years of age, 37% had
an SBA titer ≥8 a median of 1.8 years after immunization (26). In a further study, 987 children who had received 1 dose of MCC at age 6 to 15 years were followed up for a median of five years after immunization. Among these children 84% had an SBA titer ≥8 (27). This is slightly higher than observed in our study, consistent with our study being an unselected population sample that will have included some unvaccinated children. These results further highlight the importance of age at immunization in determining antibody persistence.

In adults aged 25 years and older, the percentage of sera with SBA titers ≥8 was similar in the pre- and post-vaccine era, with the exception of those aged 65 years and older, where significantly fewer achieved protective SBA titers post-vaccination. As shown with Hib, the reduction in natural boosting due to lower transmission after vaccine introduction could increase susceptibility in unvaccinated adults (18). However, since these samples were collected in 2000, less than one year after vaccine introduction, it seems unlikely that such an effect would be observed so quickly, and the reasons for this difference are unclear.

The SBA assay is the accepted surrogate of protection for meningococcal disease (3), but examination of serogroup-specific IgG levels can also be informative. In our study, serogroup C specific IgG GMC followed a similar age-specific pattern to SBA in general, with higher GMC observed in infants, comparatively lower GMC in 1-9 year olds, and then higher concentrations in teenagers, consistent with age at and time since MCC vaccination. The low levels of serogroup specific W-135 and Y IgG are unsurprising, since these serogroups are not common among cases (8) or carriers (16) in the UK. We noticed a steep increase in serogroup A specific IgG at the age of 1 year, with no evidence of maternal immunity in infants. Thereafter, GMCs were higher for serogroup A than serogroup C (significantly so, with the exception of 12–19 year olds), despite MCC vaccination. Since serogroup A carriage
and disease is also uncommon in this setting (8,16) and the age-distribution is flatter than
would be expected from the age-specific patterns of meningococcal carriage the high GMCs
reported here may be attributable to the presence of cross-reacting antigens (9,21,25).

The sera used in this study were obtained through convenience rather than random sampling,
by collecting residual sera from routine diagnostic testing. This collection has been widely
used in epidemiologic studies (for example (6,11,20,31,33,34)). One disadvantage of this
collection is that, to preserve anonymity, few details are available regarding the individuals
from whom the sera were collected. We were unable to ascertain MCC immunization status
of the individuals in this study, so we could only infer likely vaccine coverage based on
national data. Routine MCC coverage remains high (over 90%) and coverage in the catch-up
campaign was also high (84% in children aged 12-23 months, 76% in children aged 2-5 years,
85% in school aged children) (32). Selection bias in the serum collection is thought to be
limited because all individuals in the UK have free access to comprehensive healthcare.

Studies conducted elsewhere have shown that this type of convenience sampling gives similar
results to random sampling (13).

This study shows that serologic surveillance may be a useful tool in the continued monitoring
of the MCC vaccination programme. The MCC programme has been very successful to date
in reducing morbidity and mortality from meningococcal disease. In September 2006, the UK
routine infant immunization schedule was changed (5), in response to evidence from post-
licensure surveillance studies showing that only short-term direct protection is conferred
when children are routinely immunized at 2, 3, 4 months of age with MCC (29) and Hib (23)
vaccines. Now, two doses of MCC are offered in infancy at 3 and 4 months, with a later dose
incorporated at 12 months of age (in combination with Hib vaccine), to improve the level and
duration of protection. Direct protection against meningococcal serogroup C disease is thought to depend not only on immune memory (which is induced by MCC vaccines), but also on persistence of circulating antibodies (4). The sustained high prevalence of protective titers in children immunized at older ages is reassuring. The lower prevalence of protective titers in children targeted for immunization at younger ages (either with the accelerated routine schedule, or in the 1-4 year old catch-up campaign) is of some concern, but it is important to note that the incidence of serogroup C disease remains very low. Indirect vaccine protection is clearly very important in this sustained reduction in serogroup C disease. Mathematical models suggest that herd immunity persists for several years, and that serogroup C circulation will increase only slowly after reaching its nadir (30). These predictions are consistent with post-licensure disease surveillance to date, however, these models do not account for possible introductions of more transmissible or more virulent serogroup C strains from outside of the UK. Continued high quality disease surveillance is essential, but repeat seroprevalence surveys will also be useful to inform vaccine policy, in order to identify susceptible cohorts, should herd immunity wane.
ACKNOWLEDGEMENTS

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Table 1: Numbers of serum samples analyzed for serum bactericidal and serogroup-specific IgG antibodies per age group from the Health Protection Agency Seroepidemiology collection for England 2000-2004. (Note that all samples in under 20 year olds are from 2001-2004)

<table>
<thead>
<tr>
<th>Age group</th>
<th>SBA</th>
<th>Serogroup specific IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 6 months</td>
<td>49</td>
<td>48</td>
</tr>
<tr>
<td>6 -11 months</td>
<td>35</td>
<td>38</td>
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<td>1 year</td>
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<td><strong>Total</strong></td>
<td><strong>2,391</strong></td>
<td><strong>2,658</strong></td>
</tr>
</tbody>
</table>
Figure 1: Comparison of the percentage of sera with serogroup C SBA titers ≥8 (with 95% confidence intervals) by age in the pre- and post-vaccine era.

Figure 2: Percentage of sera with serogroup C SBA titers ≥8 (with 95% CI) by age targeted for vaccination, vaccine schedule and time since vaccination.

Footnote for figure 2

* for the cohorts eligible for routine infant immunization, the columns reflect an offer of vaccination within 18 months and more than 18 months ago respectively

Figure 3: Serogroup specific IgG Geometric Mean Concentrations in µg/mL against serogroups A, C, W-135 and Y, by age-group, in samples collected between 2000 - 2004.
Within 2 years of vaccination □ More than 2 years after vaccination

% with SBA titer ≥8

<1 year, routine *
<1 year, routine or 2 dose catch-up
1 year, catch-up
2-4 years, catch-up
5-9 years, catch-up
10-14 years, catch-up
15-17 years, catch-up

age at vaccination/ schedule