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Kinetics of Meningococcal Serogroup C-Specific Functional Antibody Levels Up to 15 Years after a Single Immunization with a Meningococcal Serogroup C Conjugate Vaccine during Adolescence

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ABSTRACT Adolescent vaccination is now considered the key factor for offering direct protection against meningococcal disease but also for reducing carriage and transmission and, in this way, establishing herd protection. This study estimated age-dependent patterns in functional meningococcal serogroup C (MenC) antibody kinetics after primary MenC conjugate (MenCC) vaccination in adolescents. Serum samples (n = 1,676) were drawn from 2006 to 2011 from individuals aged 9 to 18 years at the time of primary MenCC vaccination in 2002. Functional antibody levels were measured with a serum bactericidal antibody assay (SBA) using rabbit complement. SBA titers gradually declined with time. Up to 9 years after primary vaccination, SBA titers were estimated to be higher in individuals who were aged 13 to 18 years at priming than in those who were aged 9 to 10 years at priming. Based on a linear mixed model, the higher functional antibody levels with age seem to be due to the achievement of higher peak levels upon vaccination rather than to lower rates of decline. It is estimated that 35 to 50% of individuals who received a single primary MenCC vaccination at an age of 9 to 18 years in 2002 will still have sufficient protective antibody levels 15 years later. Using a linear mixed model based on cohort data for a single dated serum sample per person, we were able to estimate the level of protection against MenC up to 15 years after a single vaccination. The current study shows that analysis of antibody kinetics can be done using cross-sectional serology data and is therefore relevant for future serosurveillance studies.

KEYWORDS Neisseria meningitidis, adolescent, meningococcal serogroup C conjugate vaccine, long-term, antibodies

Invasive meningococcal disease is a severe and life-threatening disease with a mortality rate of 5 to 10% (1) and severe sequelae in ~10% of survivors. In response to an increase in meningococcal serogroup C (MenC) disease around 2000, several countries included MenC conjugate (MenCC) vaccination in their national immunization programs (NIPs). The MenCC vaccine (MenC conjugated to tetanus toxoid) was implemented as part of the Dutch NIP in 2002, as a single vaccination for all children aged 14 months. In addition, between June and November 2002, all children between 1 and 19 years of age were offered a catch-up vaccination. The vaccination coverage of this
catch-up program was 94% (2). After 2002, the incidence of MenC disease in the Netherlands dropped markedly among both vaccinated and unvaccinated populations (3). This phenomenon, referred to as herd protection, was also seen in the UK, where the MenCC vaccine was introduced in 1999 in conjunction with a nationwide catch-up campaign (4, 5). Results from a large carriage study in the UK showed that MenCC vaccines not only provide individual immunity against MenC disease but also reduce MenC carriage (6). Reduction of MenC carriage in adolescents and young adults—the age groups with the highest carriage levels (7)—is now considered the main determinant for the induction of herd protection (8).

Several postimplementation surveillance studies previously displayed an age-related effect on the persistence of MenC-specific antibody levels upon MenCC vaccination. In children aged ≥5 years, MenCC vaccination induced high MenC-specific antibody levels and good persistence (9–11), while MenC-specific antibody levels in infants and toddlers appeared to decline rapidly after one or multiple MenCC vaccinations (9, 12–14). Vaccination programs that include MenCC vaccination(s) only for infants and/or toddlers—like the current Dutch NIP—therefore fail to establish long-term individual protection. Importantly, though, results from a UK postimplementation surveillance study in 2009 indicated that the incidence of MenC disease in the UK has hardly been affected by the short duration of individual protection in infants but is mainly influenced by the herd effects induced by the catch-up campaign, i.e., large-scale reductions of MenC carriage and thereby transmission (15). In order to sustain direct protection and herd effects, it might be sensible to add an adolescent booster to the Dutch NIP program as recommended in several countries. To date, the incidence of MenC disease in the Netherlands is still low, and it is unknown how long herd protection will last. With the catch-up cohort aging, and in the absence of adolescent booster vaccination, herd protection will likely fade away. Long-term follow-up of MenC-specific antibody levels in the catch-up cohort—particularly the adolescents—in addition to close surveillance of the incidence of MenC disease may provide insight into the level of protection required to maintain herd protection.

In the current study, we assessed MenC-specific functional antibody levels in serum samples that were drawn between 2006 and 2011 from individuals who were primed with a single MenCC vaccine at 9 to 18 years of age during the catch-up campaign. Results of these measurements were used to estimate age-dependent kinetics of MenC-specific functional antibody levels and the proportion of protected individuals up to 15 years after a single primary MenCC vaccination.

RESULTS

MenC-specific SBA titers 4 to 9 years after MenCC vaccination. In total, 1,676 samples from individuals aged 9 to 18 years at the time of primary MenCC vaccination in 2002 were used for the analyses (Table 1). Serum bactericidal antibody assay (SBA) titers in 2006 and 2007 have been described previously (9). For the current analysis, the results from 2006 were used separately from the results from 2007. SBA geometric mean titers (GMTs) 4 years after priming (2006) ranged from 48 (95% confidence interval [CI], 27 to 84) for the group vaccinated at the age of 9 to 10 years to 323 (95% CI, 163 to 640) for the group vaccinated at 17 to 18 years of age (Table 1). The proportions of individuals with SBA titers of ≥8 ranged from 82% to 95% for these age groups (Table 1; Fig. 1). Between 2008 and 2011, SBA GMTs decreased gradually for all age groups immunized at 9 to 18 years of age. In 2011, the GMTs ranged from 9 to 41, and the proportions of individuals with SBA titers of ≥8 ranged from 30% to 53% (Table 1; Fig. 1). The proportions of individuals with SBA titers of ≥128 in 2011 ranged from 21% to 44% (Table 1).

Age-dependent kinetics of MenC-specific SBA titers after MenCC vaccination. Individual SBA titers from the samples collected between 2008 and 2011, together with previous results from 2006 and 2007, were used to estimate the age-specific decay in SBA titers in the years following primary MenCC vaccination. Results of the regression model showed higher population mean SBA titers throughout the first 9 years after
primary vaccination for those aged 13 to 14 and 17 to 18 years at the time of priming than those for individuals aged 9 to 10 years at the time of priming (Fig. 2). These higher SBA titers appeared to be due to an age-dependent difference in initial peak SBA titers achieved after vaccination, with the highest SBA titers observed for those vaccinated at an older age according to the model (Fig. 3A). In contrast, the rate of antibody decay decreased slightly with increasing age at vaccination (Fig. 3B). Ten years after primary MenCC vaccination, the mean SBA titers were estimated to be fairly similar across the age groups (Fig. 2).

Estimated proportion of protected individuals up to 15 years after priming. Based on the estimations of the regression model, virtually all individuals aged 9 to 18 years had developed SBA titers above the correlate of protection (SBA titer of ≥8) upon primary MenCC vaccination in 2002. By extrapolating the age-dependent patterns, it is estimated that the proportions of individuals with SBA titers of ≥8 at 15 years postpriming will range from 35 to 50% (Fig. 4). Notably, the proportion of individuals with SBA titers above the correlate of protection mainly decreased 5 to 10 years after priming, whereas the model estimated a smaller reduction of the proportion of protected individuals 10 to 15 years after priming.

### TABLE 1 Age groups and their SBA titers in this study

<table>
<thead>
<tr>
<th>Age group at yr of primary MenCC vaccination (2002) and parameter</th>
<th>Value for indicated yr of blood samplinga</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
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<tr>
<td>9–10 yr</td>
<td>Age (yr)</td>
<td>13–14</td>
<td>14–15</td>
<td>15–16</td>
<td>16–17</td>
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<td>18–19</td>
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<td>n</td>
<td>38</td>
<td>19</td>
<td>NA</td>
<td>NA</td>
<td>47</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>SBA GMT (95% CI)</td>
<td>47.8 (27.2–84.1)</td>
<td>32.0 (13.2–77.7)</td>
<td>NA</td>
<td>NA</td>
<td>22.4 (10.5–47.6)</td>
<td>9.1 (5.5–15.3)</td>
</tr>
<tr>
<td></td>
<td>% of individuals with SBA titer of ≥8 (95% CI)</td>
<td>82 (66–92)</td>
<td>68 (44–87)</td>
<td>NA</td>
<td>NA</td>
<td>51 (37–65)</td>
<td>30 (21–40)</td>
</tr>
<tr>
<td></td>
<td>% of individuals with SBA titer of ≥128 (95% CI)</td>
<td>47 (32–64)</td>
<td>26 (10–50)</td>
<td>NA</td>
<td>NA</td>
<td>32 (20–47)</td>
<td>21 (14–31)</td>
</tr>
<tr>
<td>11–12 yr</td>
<td>Age (yr)</td>
<td>15–16</td>
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<td>18–19</td>
<td>19–20</td>
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<td>48</td>
<td>56</td>
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<tr>
<td></td>
<td>SBA GMT (95% CI)</td>
<td>118.0 (58.6–237.5)</td>
<td>100.1 (50.0–200.5)</td>
<td>71.1 (31.7–159.5)</td>
<td>36.0 (21.0–61.7)</td>
<td>42.5 (26.2–69.0)</td>
<td>10.6 (11.9–35.5)</td>
</tr>
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<td></td>
<td>% of individuals with SBA titer of ≥8 (95% CI)</td>
<td>91 (71–100)</td>
<td>100 (80–100)</td>
<td>84 (66–94)</td>
<td>67 (52–79)</td>
<td>58 (48–68)</td>
<td>69 (59–78)</td>
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<td>% of individuals with SBA titer of ≥128 (95% CI)</td>
<td>56 (39–72)</td>
<td>52 (34–69)</td>
<td>52 (38–66)</td>
<td>38 (28–48)</td>
<td>40 (31–50)</td>
<td>33 (24–43)</td>
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<td>13–14 yr</td>
<td>Age (yr)</td>
<td>17–18</td>
<td>18–19</td>
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<td>21–22</td>
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<td>n</td>
<td>23</td>
<td>21</td>
<td>96</td>
<td>99</td>
<td>101</td>
<td>86</td>
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<tr>
<td></td>
<td>SBA GMT (95% CI)</td>
<td>173.0 (81.4–367.6)</td>
<td>264.6 (130.0–538.6)</td>
<td>113.2 (66.8–192.0)</td>
<td>77.7 (32.2–103.4)</td>
<td>70.8 (42.1–119.2)</td>
<td>53 (43–64)</td>
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<td></td>
<td>% of individuals with SBA titer of ≥8 (95% CI)</td>
<td>78 (56–92)</td>
<td>86 (63–97)</td>
<td>86 (63–97)</td>
<td>58 (48–68)</td>
<td>49 (40–59)</td>
<td>51 (42–61)</td>
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<td></td>
<td>% of individuals with SBA titer of ≥128 (95% CI)</td>
<td>78 (56–92)</td>
<td>86 (63–97)</td>
<td>86 (63–97)</td>
<td>58 (48–68)</td>
<td>49 (40–59)</td>
<td>51 (42–61)</td>
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<td>15–16 yr</td>
<td>Age (yr)</td>
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<td>99</td>
<td>100</td>
<td>81</td>
<td>51</td>
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<tr>
<td></td>
<td>SBA GMT (95% CI)</td>
<td>208.8 (82.4–529.3)</td>
<td>309.8 (189.5–506.3)</td>
<td>161.1 (94.6–274.5)</td>
<td>161.1 (94.6–274.5)</td>
<td>161.1 (94.6–274.5)</td>
<td>161.1 (94.6–274.5)</td>
</tr>
<tr>
<td></td>
<td>% of individuals with SBA titer of ≥8 (95% CI)</td>
<td>98 (85–100)</td>
<td>100 (80–100)</td>
<td>81 (72–88)</td>
<td>62 (52–71)</td>
<td>70 (61–79)</td>
<td>53 (43–64)</td>
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<tr>
<td></td>
<td>% of individuals with SBA titer of ≥128 (95% CI)</td>
<td>76 (50–93)</td>
<td>78 (62–88)</td>
<td>61 (51–70)</td>
<td>50 (40–60)</td>
<td>59 (48–70)</td>
<td>33 (22–47)</td>
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<td>87</td>
<td>72</td>
<td>28</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>SBA GMT (95% CI)</td>
<td>322.5 (162.6–639.6)</td>
<td>192.4 (99.8–371.1)</td>
<td>154.9 (82.3–291.4)</td>
<td>42.1 (20.7–85.9)</td>
<td>49.2 (19.8–122.1)</td>
<td>38.7 (9.1–156.4)</td>
</tr>
<tr>
<td></td>
<td>% of individuals with SBA titer of ≥8 (95% CI)</td>
<td>95 (74–100)</td>
<td>97 (83–100)</td>
<td>75 (64–83)</td>
<td>54 (42–66)</td>
<td>68 (48–83)</td>
<td>50 (29–71)</td>
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<tr>
<td></td>
<td>% of individuals with SBA titer of ≥128 (95% CI)</td>
<td>86 (63–97)</td>
<td>65 (47–79)</td>
<td>63 (53–73)</td>
<td>46 (35–58)</td>
<td>50 (32–69)</td>
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*aNA, not available.*
DISCUSSION

The present study presents results of an antibody kinetics study of MenC-specific functional antibody levels after a single primary MenCC vaccination in individuals aged 9 to 18 years at the time of priming in 2002. Specifically, we were able to study antibody
kinetics by using a set of time-stamped serum samples. The proportion of individuals with SBA titers of $\geq 8$ gradually declined with time. Linear mixed-model analysis showed higher functional antibody levels up to 9 years after primary vaccination for individuals aged 13 to 18 years at priming than for those aged 9 to 10 years at priming. According to the model, these higher levels appeared to be due to the achievement of higher initial peak functional antibody levels upon vaccination rather than to lower rates of decline. Furthermore, the model estimated that 35 to 50% of individuals who received a single primary MenCC vaccination at an age of 9 to 18 years in 2002 will still have sufficient protective antibody levels 15 years later.

Based on the measured values, we found that SBA titers after primary MenCC vaccination continued to decrease with time. This ongoing decrease was expected

FIG 3 Peak SBA titers (A) and decay rates of SBA titers (B) after the single primary MenCC vaccination in 2002 in individuals aged 9 to 18 years. Each set of lines represents the model-estimated population mean and 95% CI. The peak SBA titer upon vaccination increased with age and was highest in those primed at the age of 17 to 18 years. The rates of antibody decay appeared to be similar across ages.

FIG 4 Model-estimated proportions of protected individuals (SBA titers of $\geq 8$) up to 15 years after a single primary MenCC vaccination in 2002, at an age of 9 to 18 years. It is estimated that 15 years after the primary MenCC vaccination, 35 to 50% of this population will have SBA titers of $\geq 8$. 
because, to date, the incidence of MenC disease is still very low (16), suggesting that the samples used in this study were collected during a period with low MenC carriage and therefore a low chance of natural boosting of MenC-specific immunity (3). In coherence, a recently published carriage study from the UK found very low levels of MenC carriage up to 13 years after the introduction of MenC vaccination (17). It is assumed that MenC-specific functional antibody levels will continue to decrease with time and that herd effects will eventually fade away. In anticipation of this waning immunity, an adolescent meningococcal booster vaccination was implemented in the UK, a measure that will likely secure herd protection. Due to the very low incidence of MenC disease after introduction of the MenCC vaccine in the Netherlands, only a single dose of MenCC is still used in the current Dutch NIP, for children of 14 months of age.

The proportions of individuals with SBA titers of ≥8 in this study ranged from 54% to 62% in 2009 (7 years postpriming), from 51% to 74% in 2010 (8 years postpriming), and from 30% to 53% in 2011 (9 years postpriming). The slightly deviating proportions for 2010 are probably a result of the pseudolongitudinal follow-up in this study and may reflect the presence of a wide distribution of SBA titers in the general population. This variation was incorporated into the model, and the proportions of individuals with SBA titers of ≥8 in 2012 (10 years postpriming) were estimated to range from 50% to 62%. This is comparable to results from the UK reported in 2012 by Ishola et al., who found SBA titers of ≥8 in 56% of individuals 10 years after priming at an age of 10 to 17 years. Estimated vaccine coverage in the UK for this population was 60 to 88% (10). Notably, De Whalley et al. recently reported SBA titers of ≥8 for over 90% of individuals 11 years after priming at a median age of 10.6 years (18). These results were generated in a randomized trial, i.e., a population with 100% vaccine coverage. Considering the vaccine coverage of 94% during the catch-up campaign in the Netherlands (2), the estimated proportion of individuals with SBA titers of ≥8 in the current study may even be a slight underestimation. It has to be noted, however, that the SBA titers were measured with rabbit complement, which may result in higher titers than those obtained with human complement (19). Therefore, we also analyzed functional antibody titers by using the more conservative correlate of protection of an SBA titer of ≥128. According to the model, the proportions of individuals with SBA titers of ≥128 in 2012 (10 years postpriming) were estimated to range from 22% to 35%. Ongoing surveillance is required to maintain insight into MenC-specific immunity in the population.

We found higher mean SBA titers for up to 9 years post-primary MenCC vaccination among individuals aged 13 to 18 years than among those aged 9 to 10 years at priming. This difference was caused—as suggested by the results of the model—by higher peak SBA titers with age upon primary MenCC vaccination rather than by a difference in decay rates. A similar conclusion was drawn by Ishola et al. based on results of two cross-sectional surveys, performed 4 and 10 years after the introduction of the MenCC vaccine in the United Kingdom (10). Our results are also in line with results from Snape et al. (11), who found a clear increment of protective antibody levels for up to 5 years after vaccination between children aged <10 years and children aged ≥10 years at primary MenCC vaccination. An age beyond 10 years at priming made little further difference in the level and persistence of protective antibodies (11). It is thought that this age-related difference in persistence of MenC-specific functional antibody levels after primary MenCC vaccination is due either to maturation of the immune system or to previous exposure of older individuals to the meningococcus (11). The latter suggests that the primary MenCC vaccination in adolescents boosted naturally acquired immunity. Our finding of higher (estimated) peak functional antibody levels upon primary MenCC vaccination in the older age groups may support this suggestion. Meningococcal carriage levels increase steeply during adolescence (7), and boosting of preexisting immunity induces higher peak antibody levels (20, 21). Nevertheless, a dominant role for immune maturation cannot be excluded. Importantly, considering the current absence of natural exposure, ongoing surveillance and vaccination studies are warranted to determine the effect of this absence on the persistence of MenC-
specific antibody levels after (booster) vaccination in adolescents and its impact on maintenance of herd protection.

The proportion of individuals with SBA titers of $\geq 8$ mainly decreased from 5 to 10 years after primary MenCC vaccination according to the model, whereas a smaller reduction of this proportion from 10 to 15 years after priming was estimated. This suggests that a number of individuals developed very high SBA titers in response to the primary MenCC vaccination in 2002 that will remain elevated for a prolonged period (more than 15 years). Indeed, in 2011, over one-third of the study population had SBA titers of $\geq 128$ (and $\sim 75\%$ of the individuals had SBA titers of $\geq 8$). These individuals possibly had preexisting naturally induced immunity. Part of the cohort vaccinated at 9 to 18 years of age in 2002 has now reached the reproductive age. It is likely that females with remaining high SBA titers will pass these antibodies on to their babies, which may be contributing to the ongoing low incidence of MenC disease among infants in the Netherlands. Nevertheless, as the catch-up cohort ages and SBA titers continue to decline, all herd effects will eventually fade away, and recirculation of MenC seems inevitable.

A limitation of the current study is that the results were not based on longitudinal follow-up data. Instead, we used sets of serum samples from different individuals to attain a pseudolongitudinal cohort. Our finding of higher SBA GMTs in 2010 than in 2009 particularly illustrates the nonlongitudinal follow-up. However, this finding may reflect the presence of a very wide distribution of SBA titers in the general population, which might have been missed if an actual longitudinal cohort was used. Notably, despite the wide range of SBA titers in our study population, the model still allowed estimation of the average decay of SBA titers in the population, as illustrated by the estimated decay curves in Fig. 2. Notwithstanding the considerable uncertainty in the shape of individual decay curves, our analysis demonstrated that a set of time-stamped serum samples may allow study of antibody kinetics. As mentioned above, SBA titers measured with human complement may result in lower titers than those obtained with rabbit complement. Nevertheless, antibody decay rates were expected to be similar when human complement was used in the SBA for all measurements. Another limitation is that we had no samples available from the period of 0 to 4 years after primary vaccination. The part of the estimated decay curves that represents this time frame should therefore be interpreted with caution. However, additional analysis using a power function decay model, which assumes that antibody decay rates could have been higher shortly after the primary vaccination, also estimated that the oldest age group showed the highest initial peak SBA titers compared to the younger age groups (22; data not shown). Importantly, the antibody decay within the time frame for which we had samples available, i.e., 4 to 9 years after primary MenCC vaccination, was presumably in a steady state. Therefore, the estimation of the proportion of individuals with SBA titers of $\geq 8$ 15 years after primary MenCC vaccination is likely reliable.

To conclude, after a single primary MenCC vaccination in adolescents aged 9 to 18 years in 2002, MenC-specific functional antibody levels gradually but continuously declined with time. However, a substantial part of this population has maintained high SBA titers for an extended period, possibly due to the presence of preexisting immunity prior to priming. We estimated that 15 years after primary MenCC vaccination, 35 to 50% of the population primed at an age of 9 to 18 years will still have SBA titers above the correlate of protection. The current study shows that modeling of antibody kinetics based on just one serum sample per person can be used among cross-sectional cohorts and is relevant for serosurveillance studies.

**MATERIALS AND METHODS**

**Study design and collection of samples.** We performed a retrospective antibody decay study by assessing MenC-specific functional antibody levels in serum samples from separate cohorts categorized according to the year of blood sampling. Included (single) serum samples were previously drawn from individuals who were between 9 and 18 years of age in 2002, the year of primary MenCC vaccination.
Inclusion of samples was random and based solely on the age of the individual in 2002. Samples were anonymous, and vaccination history was not known. A single MenCC vaccination in 2002 was assumed based on the high vaccination coverage of the Dutch catch-up campaign (94%) (2).

Samples collected between 2008 and 2011 were randomly selected from the Biobank of the University Medical Center Utrecht. Approval for the use of these samples was obtained from the Scientific Advisory Board Biobank (Wetenschappelijke Adviesraad Biobank [WARBI]) of the UMC Utrecht in March 2011. For the years 2008 and 2009, no samples were available for individuals aged 9 to 10 years at the time of primary vaccination in 2002. To expand the amount of data available for the estimation of the age-dependent kinetics of MenC-specific functional antibody levels in the years following primary MenCC vaccination, we included the results on MenC-specific functional antibody levels of individuals aged 9 to 18 years in 2002 from the Dutch nationwide cross-sectional serosurveillance study that was performed by our institute between February 2006 and June 2007 (ISRCTN 20164309) (23). Results from this nationwide study on MenC-specific functional antibody levels in 2006 and 2007 in individuals of all ages have been published previously (9). For the current study, the results of 2006 and 2007 were used separately in the analysis.

**SBA.** The levels of MenC-specific functional antibody were determined with a serum bactericidal antibody assay (SBA) using meningococcal serogroup C target strain C11 and baby rabbit complement (Pelfreez) as an exogenous complement source (24). The SBA titer was expressed as the reciprocal of the final serum dilution yielding ≥50% killing after 60 min, with a titer of ≥8 used as a correlate of protection (25, 26). In addition, functional antibody titers were also analyzed with a threshold of ≥128, which is the more conservative correlate of protection when rabbit complement is used (19). Samples were analyzed in triplicate, and the mean value was used for statistical analysis. SBA titers below the lowest limit of detection (<4) were assigned a value of 2 for statistical purposes.

**Statistical analysis of measured SBA titers 4 to 9 years post-primary MenCC vaccination.** SBA titers of samples from 2006 to 2011 were logarithmically transformed, and geometric mean titers (GMTs) with 95% confidence intervals (95% CI) were calculated for 5 different age groups based on age at the time of primary vaccination in 2002: 9 to 10 years, 11 to 12 years, 13 to 14 years, 15 to 16 years, and 17 to 18 years. Proportions (and 95% CI) of participants with SBA titers of ≥8 were calculated using the Agresti-Coull method (27).

**Modeling of antibody kinetics 0 to 15 years post-primary MenCC vaccination.** SBA data were analyzed as a (pseudo)longitudinal data set with antibody decay, as follows: y(t,age) = a(age) + b(age)t, where y(t,age) is the log functional antibody titer as a function of time since vaccination and age at vaccination, assuming that antibody decay is exponential, with the age-dependent initial (log) functional antibody titer a(age) and the age-dependent decay rate b(age). Age dependence was modeled as penalized (P-)splines (28), as follows: a(age) = BXb + BZc, and b(age) = BXB + BZd, where B is an n × k cubic B-spline basis with k equally spaced knots, X is a k × d matrix such that Xb is a polynomial of degree d − 1, and Z = D®(DD®)−1 = a k × (k − d) matrix, with D being a (k − d) × k difference matrix of order d. β is a vector of length d, and b is a vector of length (k − d). In this study, k = 6, using 6 knots for equally distributed age groups between 5 and 25 years, and d = 2, penalizing second-order curvature. Each observation was treated as a single measurement from the longitudinal response of a subject, and a mixed model was fitted to allow for individual variation and to estimate population responses. Parameters were estimated in a Bayesian framework through Gibbs sampling using JAGS (29) via rjags on R (30). Three parallel Markov chains were run, each with a burn-in of 100,000 iterations, followed by 100,000 iterations that were thinned by a factor of 100, yielding a posterior sample of 3 times 1,000 iterations.

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