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# **Impaired antibody response to conjugated meningococcal serogroup C vaccine in asplenic patients**

Running head: MenC conjugated vaccine in asplenia

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## Abstract

**PURPOSE** To determine the quantity and quality of antibodies against the meningococcal serogroup C (MenC) conjugated vaccine in asplenic patients

**PATIENTS AND METHODS** In 116 asplenic patients, antibody concentrations (IgG) were measured against meningococcal serogroup C before and after immunisation. Of MenC-specific IgG, both antibody avidity and subclasses of IgG1 and IgG2 were determined.

**RESULTS** Mean MenC IgG concentration rose from 0.16 µg/mL prior to vaccination to 3.69 µg/mL 3 weeks post-vaccination, with 67% of patients reaching the threshold of  $\geq 2.0$  µg/mL. Mean IgG concentration at 35 weeks post-vaccination was 3.10 µg/mL. IgG2 concentrations increased more than IgG1. Marginal avidity maturation was seen. Hypo-responders to the first MenC vaccine (IgG anti-MenC  $\leq 2.0$  µg/mL) were offered a booster dose. After revaccination, 59% reached the chosen IgG threshold. The IgG concentration rose from 0.29 to 1.12 µg/mL, with an increase in the IgG1/IgG2 ratio. Avidity indices remained below 33%.

**CONCLUSIONS** In asplenic patients the quantity and quality of antibodies produced after one dose of conjugated MenC vaccination is lower than observed in previous studies in healthy adults. Booster vaccination does lead to a rise in IgG GMC's, but does not lead to higher avidity of antibodies.

**Keywords:** meningococcal serogroup C, conjugated vaccines, asplenia, avidity, IgG subclasses

## Introduction

Functional or anatomical asplenic patients are at increased risk of a severe overwhelming post splenectomy infection (OPSI) with encapsulated bacteria. After *Streptococcus pneumoniae* as the major cause, one of the other prevalent causative organisms is *Neisseria meningitidis*. [1-3] To reduce the incidence of these infections in asplenic patients, a vaccination schedule including *N. meningitidis* is strongly recommended. [4]

Of the known meningococcal groups, 5 groups (A, B, C, Y and W135) are responsible for more than 95% of invasive disease. [5] In 2002, after an outbreak of MenC infection, a national vaccination campaign was performed in the Netherlands in which all persons 1-18 years of age were vaccinated against MenC. Since then, due to vaccination and herd immunity, the circulation of MenC is very low. [6]

Data on the ability of asplenic individuals to establish an adequate antibody response to vaccination with polysaccharide vaccines are conflicting. The response depends among others on the reason for asplenia and on the vaccine that is used (polysaccharide versus conjugated). [7-10] Immunisation with meningococcal polysaccharide vaccines was not recommended for patients with asplenia due to the short duration of protection and the absence of protection against the most common serogroup B. [2,11] With the introduction of conjugated vaccines, inducing long-term immunity, vaccination of asplenic patients with meningococcal conjugated vaccines now is recommended. [11,12]

Beside increase in antibody concentration as a surrogate marker of protection offered by the MenC vaccine, antibody avidity can be determined. Antibody avidity is a biomarker, reflecting the generation of immunological memory after immunisation. [13-15]

Antibody avidity is defined as the overall antigen-binding capacity of polyclonal antibodies with different affinities. [13,14,16,17] Polysaccharide conjugate vaccines have the ability to elicit higher avidity antibodies with increased functional activity than do plain polysaccharide

vaccines.[14] The magnitude of the avidity index is dependent on the type of conjugate vaccine.[18]

In this study, the efficacy of a MenC conjugated vaccine in terms of increase in antibody concentration after vaccination (quantity) and the avidity of the antibodies (quality) is determined in adult, asplenic patients.

## Patients and methods

### Patients

In 2006 and 2007, patients were included in a study to evaluate the vaccination coverage of adult asplenic patients in the Utrecht area of the Netherlands.[19] This study was approved by the local institutional ethics committee and all patients gave written informed consent. When applicable, patients were vaccinated with conjugated pneumococcal, *Hib* and meningococcal vaccines according to the institutions protocol and serum samples were taken pre- and post-vaccination to determine antibody concentrations. Results of the pneumococcal and *Hib* vaccines will be reported separately (Meerveld *et al.*, manuscript submitted).

### Vaccination protocol

Patients were vaccinated against *N. meningitidis* when they were never vaccinated before, when vaccination status against the meningococcus could not be retrieved or when vaccination with a polysaccharide meningococcal vaccine occurred more than three years ago. A first dose of conjugate meningococcal group C vaccine was given, consisting of MenC polysaccharide covalently coupled to tetanus toxoid (NeisVac-C<sup>®</sup>, Baxter AG, Vienna, Austria). Blood samples were drawn pre-immunization, 3 weeks and 35 weeks post-immunization. Serum samples were stored at -20°C until use. When antibody concentrations after the first vaccination remained below 2 µg IgG/mL in patients (so-called hypo-responders), a second dose of conjugate MenC vaccine was given.

### Laboratory determinations

Meningococcal antibody concentrations were measured against serotype C by multiplex immunoassay (MIA) as described previously.[20-22] All antibody concentrations were log-transformed and geometric mean concentrations (GMC) were calculated. Seroconversion was defined as a  $\geq$  fourfold increase in antibody concentration after vaccination. The chosen

anti-meningococcal antibody threshold for correlation with long-term protection was  $\geq 2.0$   $\mu\text{g/mL}$ .

MenC-specific IgG1 and IgG2 subclasses were measured by a modification of the MIA as described above.[23] Previously assigned concentrations of meningococcal IgG1, and IgG2 antibodies were used in this assay.[24,25]

Avidity of MenC-specific IgG antibodies was assessed by using a modification of the MIA for determining MenC-specific IgG.[20,21] Serum samples with an IgG concentration of  $\geq 0.25$   $\mu\text{g/mL}$  were diluted to an antibody concentration of 25 ng/mL in a buffer composed of 25% antibody depleted human serum (Valley Biomedical, USA). Ammonium thiocyanate ( $\text{NH}_4\text{SCN}$ ; Sigma-Aldrich, St. Louis, MO) was added to a final concentration of 0.5 M in order to dissociate low-avidity antigen-antibody binding.[26] After incubation of polysaccharide-conjugated-beads with serum, 0.5M  $\text{NH}_4\text{SCN}$  was added for 10 minutes exactly. Beads were washed, *R*-phycoerythrin(*RPE*)-conjugated goat anti-human IgG (gamma chain specific) (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) was added and incubated for a further 30 minutes. After a final wash, the plate was read on a Luminex-100 system (Bio-plex; Bio-Rad Laboratories, Hercules, CA). Data are expressed as the avidity index (AI) which is the percentage of antibodies that remained bound to the MenC polysaccharide-conjugated beads after treatment with  $\text{NH}_4\text{SCN}$  and was calculated as follows:  $\text{AI} = (\text{amount of IgG bound in the presence of } 0.5 \text{ M } \text{NH}_4\text{SCN}) / (\text{amount of IgG bound in PBS}) \times 100$ . Sera were categorized into high, intermediate and low avidity, based on the AI: high, 100-67%; medium, 66-34%; and low, 0-33%.

### Statistical analysis

Comparisons of GMC before and after vaccination (paired samples) and comparisons of antibody avidity before and after vaccination were performed using the paired samples T-test. Independent sample T-test and Chi-square were used when appropriate. A *p*-value of  $\leq$

0.05 was considered statistically significant. In case of subset analysis, in small groups the Wilcoxon signed rank test was used.

For statistical analysis, the Statistical Package for the Social Sciences (SPSS) for Windows® software, version 15.0 (SPSS Inc., Chicago, IL) was used.



## Results

We included 184 asplenic patients in this study. Previously, we have described the characteristics of the majority of this group.[19] In 117 patients a MenC conjugated vaccine was indicated. Samples after vaccination, of all but one patient, were available and these 116 sera were analysed in this study. Baseline characteristics of the patients are given in Table 1. All but one patient underwent a splenectomy, the prevalent reason being a trauma (30%). One patient was considered to be functional asplenic, because of irradiation of the spleen for Hodgkin's disease.

Seventeen (15%) patients had previously received a meningococcal vaccination, either MenA,-C,-W135 and-Y polysaccharide vaccine (n=6), MenA,-C polysaccharide vaccine (n=4) or an otherwise not-specified meningococcal vaccine (n=7). The median time since the last vaccination was 4 years (range 2-6 years).

### Meningococcal antibody concentrations

In asplenic patients, MenC geometric mean antibody concentrations (GMC) rose from 0.16 µg/mL prior to vaccination to 3.69 µg/mL three weeks post-vaccination (Figure 1). For MenC, trauma patients had significantly higher post-vaccination antibody concentrations than the other asplenic patients ( $p < 0.05$ ). We observed no statistically significant influence of age at splenectomy, age at vaccination or time from splenectomy to vaccination on the antibody concentrations of MenC-specific IgG. Previous meningococcal vaccination had a statistically significant influence on the MenC GMC's pre- and post-vaccination ( $p < 0.05$ ). GMC pre-vaccination in previously vaccinated patients was 1.6 µg/mL (95% CI 0.5-5.7) versus 0.1 µg/mL (95% CI 0.1-0.2) in patients not vaccinated before. For GMC post-vaccination, these data were 10.7 µg/mL (95% CI 4.8-24.0) and 3.1 µg/mL (95% CI 2.1-4.8), respectively. GMC's post-vaccination were 10% higher in patients receiving the MenC vaccine alone

compared to patients receiving concomitant pneumococcal and/or *Hib* vaccines. This difference was not statistically significant.

Post-vaccination, 67% of the patients reached the threshold of 2.0 µg/mL for MenC (Table 2). Patients who were splenectomised because of trauma reached this threshold more often than other patients ( $p < 0.05$ ). Time since splenectomy did not influence seroconversion of MenC.

In 38 patients, we were able to determine meningococcal antibody concentrations up to approximately eight months after the first conjugate MenC vaccine. Mean time since vaccination was 8.3 months (range 6,5 – 10,5). GMC's for serogroup C are given in Figure 2. In this group of 38 patients, for MenC GMC pre-vaccination was 0.21 µg/mL and rose to 3.71 µg/mL post-vaccination ( $p < 0.05$ ). After eight months, a decrease in GMC to 3.10 µg/mL was observed. This decrease was not significant compared to the GMC 3 weeks post-vaccination.

Thirty eight patients (33%) did not reach adequate antibody concentrations after one dose of conjugate vaccine. Characteristics of this group of hypo-responders are given in Table 3. These hypo-responders had an adequate response to *Hib* vaccination (98% reached the threshold) and pneumococcal conjugate vaccination (93,5% reached protective antibody concentrations of 1.0 µg/mL for 5 out of 7 vaccine-serotypes after one vaccination). Of the 38 patients, 58% ( $n=22$ ) did reach seroconversion for serogroup C after the first vaccination. Because of their subnormal antibody response, a revaccination with the MenC conjugate vaccine was performed. Sera after revaccination were available of 22 patients. In this patient group, GMC pre-vaccination was 0.05 µg/mL, rising after the first vaccination to 0.29 µg/mL. After the second vaccination, the GMC of MenC PS-specific IgG rose significantly to 1.12 µg/mL (Figure 3). Thirteen (59%) patients reached the threshold of  $\geq 2.0$  µg/mL.

### **Antibody avidity**

The avidity index (AI) of the MenC antibodies was measured using a grading system to discriminate between sera with low AI (0-33%), intermediate AI (34-66%) and high AI (67-100%). Only sera that  $\geq 0.25$   $\mu\text{g/mL}$  of MenC-specific IgG were selected for AI determination. In the overall cohort, the geometric mean AI (GMAI) was 27% (95% CI 19-35%) pre-vaccination (n=48), 25% (95% CI 20-30%) 3 weeks post-vaccination (n=104) and 32% (95% CI 23-41%) 35 weeks post-vaccination (n=34), respectively. In patients who were revaccinated, the mean AI rose from 14% after the first dose of vaccine to 26% after the second vaccination (n=13;  $p < 0.01$ ). No associations were found between the AI pre- and post-vaccination and the underlying disease resulting in asplenia or previous vaccination with meningococcal vaccines.

### **IgG subclasses**

In Figure 4a, the concentrations of MenC IgG1 and IgG2 are given before, three weeks and 35 weeks after vaccination in patients with  $\geq 0.25$   $\mu\text{g/mL}$  of MenC PS-specific IgG pre-vaccination. In Figure 4b the development of the IgG1/IgG2 ratio in this patient group is given in relation to the changes in avidity indices. Great inter-individual differences between antibody responses were seen, with IgG1/IgG2 ratio's increasing in 7 patients and decreasing in 26 patients. In only 7 patients the IgG1/IgG2 ratio after one conjugated vaccination was  $>1$  and in only 2 patients the ratio changed from  $<1$  to  $>1$  post-vaccination. The mean IgG1 concentration after vaccination was 1.17  $\mu\text{g/mL}$  versus 9.52  $\mu\text{g/mL}$  IgG2. The ratio of IgG1/IgG2 did not change by vaccination (0.23 pre-vaccination, 0.25 3 weeks post-vaccination, and 0.20 35 weeks post-vaccination). After revaccination, the mean IgG1 concentration rose from 0.93 to 3.25  $\mu\text{g/mL}$  (Figure 4c). The concentration of IgG2 rose from 0.76 to 1.72. The ratio of IgG1/IgG2 was 1.43 post-vaccination, which is significantly higher ( $p < 0.01$ ) than after primary vaccination.

## Discussion

The immune response to the MenC conjugate vaccine in a group of 116 asplenic patients was found to be of poor quality and quantity. After a single dose of MenC conjugate vaccine, only 67% of the asplenic patients reached the chosen threshold antibody concentration of  $\geq 2.0 \mu\text{g/mL}$ , with an increase in GMC from  $0.16 \mu\text{g/mL}$  to  $3.69 \mu\text{g/mL}$  post-vaccination. In healthy children and adults, including our own series, 5-10 times higher GMC's were reached after one dose of this MenC conjugate vaccine. [27-30] Previously, Balmer *et al.*, using a different MenC conjugated vaccine, found three times higher GMC's in asplenic patients after vaccination compared to our asplenic patients.[31] In that study, no difference was found between IgG GMC's in asplenic patients and an age-matched control group. However, functionality of the antibodies (based on bactericidal antibody in serum (SBA)) was significantly lower in the asplenic patient group. In our asplenic patient group, the functionality of the antibodies in terms of antibody avidity was also low. As indicated above we used a different MenC conjugated vaccine (MenC coupled to tetanus toxoid (TT)) than Balmer *et al.* (CRM<sub>197</sub> conjugated). The vaccine we used has turned out to be more immunogenic in healthy subjects than other MenC conjugated vaccines. [30] Yet, in our patients lower antibody concentrations were observed than in the study of Balmer *et al.*[31] Balmer *et al.* recommend a second dose of vaccine offered to the patients if their post-vaccination concentration is below the proposed protective threshold, or routinely offering two doses of MenC conjugate vaccine. In our study, after revaccination still 41% of the revaccinated patients did not achieve protective serum levels. This percentage is in accordance with the percentage of hypo-responders found by Balmer *et al.*[31] We therefore can conclude that hypo-responsiveness to MenC remained in a considerable fraction of patients and cannot be overcome by a more potent conjugate MenC vaccine.

In healthy adults, IgG GMC's fell by 50% from 33.3 to 16.7  $\mu\text{g/mL}$  at six months after a single dose of MenC-TT vaccine.[29] In the asplenic patients of whom sera were available eight

months after vaccination, GMC dropped only slightly from 3.69 to 3.10 µg/mL. The asplenic state of the patients may be a factor which determines the only marginal decrease in GMC in the 8 months post-vaccination. In accordance with our findings, in the study of Smets *et al.*, 6 months after pneumococcal conjugate vaccination in asplenic children, GMC's had not decreased significantly.[10] High antibody concentrations months after vaccination can be explained by ongoing production of antibodies and/or lower rate of removal from the circulation. In asplenic individuals, alternative homing of peripheral B-cells takes place, with B-memory-cells homing in peripheral organs and bone marrow in stead of in the spleen. These specific niches may be favourable for long lived plasma cells. Another argument for longer persistence of antibodies in asplenic patients is the absence of the reticulo-endothelial system (RES) function of the spleen. Immune complexes can longer remain in circulation and cause prolonged B-lymphocyte activation.

Antibody persistence following pneumococcal polysaccharide or conjugate vaccination of healthy adults (50-80 years) with an intact spleen has been studied by Goldblatt *et al.*[32] One year after one dose of pneumococcal conjugate vaccine, GMC's for 4 out of the 7 pneumococcal serotypes were at least 80% of the GMC's at the peak of the response, i.e. 1 month after vaccination. It should therefore be concluded that polysaccharide (conjugated) vaccines have the ability to induce antibody levels which persist for periods up to 1 year after vaccination, in the presence or absence of a functional spleen.

### **Avidity and IgG subclasses**

It is thought that long term protection after immunisation with conjugate vaccines depends on herd immunity, persistence of anti-capsular antibodies in serum and immunological memory.[33] As a marker of immunological memory, avidity of the MenC-specific antibodies was determined.

High antibody avidity indicates high functional activity of the meningococcal antibodies.[13] In the asplenic patient group, the GMAI rose from 19% 3 weeks post-vaccination to 29% eight months after vaccination. The rise in antibody avidity is consistent with earlier studies

on affinity maturation of antibodies after conjugated vaccines.[13,15,29,34] However, in our study, most patients do not develop antibodies with intermediate or high avidity; most antibodies are of low avidity.

In adults, natural exposure to meningococci and exposure to cross-reactive agents can lead to the development of memory B-cells (priming). These memory B-cells will be stimulated after polysaccharide and conjugate vaccination, leading to higher avidity indices in adults compared to children.[17,35] For this reason, measurement of AI's in adults may be considered to be of less importance.[17,35,36] In our adult asplenic group however, most adults had low antibody avidity indices before vaccination. In this patient group therefore, an antibody AI measurement may be an adequate way of determining the development of immunological memory.

In general, IgG1 antibodies have a higher affinity than IgG2.[34] The ability to induce a IgG1 T-cell dependent (TD) response is among others dependent on age at vaccination, type of vaccine and type of adjuvant.[36-38] In the asplenic patients studied, after one meningococcal conjugate vaccination, both IgG1 and IgG2 increase, with higher concentrations of IgG2 (mean concentration 9.52 µg/mL 3 weeks after vaccination) than IgG1 (mean concentration 1.17 µg/mL). There is a large variability in the IgG1/IgG2 ratio in individual patients. This is consistent with the findings of Findlow *et al.*, who detected significant increases in IgG1 and IgG2 with large differences in IgG1/IgG2 ratio's after vaccination with one dose of quadrivalent meningococcal conjugated vaccine in healthy adults.[37] After revaccination, the amount of IgG1 in the asplenic population rises more than IgG2, suggesting that after revaccination, the T-cell dependent response is more adequate.

### **Influence of immunisation schedule**

In contrast with the study of Balmer *et al.*, in the current study a number of patients received *Hib* and pneumococcal vaccines concomitant with the MenC vaccine, dependent on the

vaccination status before start of the study.[31] The response to *Hib* vaccine was much better than to MenC vaccine; 97% of the patients reached a protective antibody concentration after vaccination (Meerveld-Eggink, manuscript submitted). Theoretically, the diminished response to MenC could be due to the simultaneous administration of different vaccines, thereby overloading the immune system. Although the MenC-specific IgG GMC post-vaccination in patients receiving concomitant vaccines was 10% lower than in patients solely immunised with the MenC vaccine, this difference was not statistically significant.

In both the current study in asplenic patients and the study of Balmer *et al.*, 15-30% of patients had received prior MenC polysaccharide vaccination. [31] These previously vaccinated patients had higher MenC GMC's pre-vaccination than patients not vaccinated with meningococcal vaccines before. In contrast to Balmer *et al.*, we found that previous MenC vaccination also lead to higher GMC's post-vaccination.

## **Conclusions**

We conclude that a single dose of MenC conjugated vaccine does not result in an adequate antibody response in 33% of asplenic patients. A second dose of MenC vaccine leads to an additional increase in antibody concentration and avidity, suggesting that asplenic patients benefit from a second dose of vaccine. This conclusion should be limited to older, asplenic patients, who did not receive MenC conjugated vaccines as part of the Dutch National Vaccination Programme. The relevance of a second dose of vaccine in countries where all children are vaccinated against MenC and circulation of MenC therefore is low, should be considered.

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## **Conflict of interest**

The authors declare that they have no conflict of interest.



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Table 1. Baseline characteristics of asplenic patients vaccinated with MenC conjugate vaccine

Patients (n)	116
Male gender	59 (51)
Reason for Sx	
Trauma	35 (30)
ITP	18 (16)
Hodgkin's disease	16 (14)
Spherocytosis	16 (14)
Other reasons	30 (26)
Functional asplenia	1 (1)
Age at Sx in years	28.3 (3-78)
Age at vaccination in years	52.5 (23-86)
Time from Sx to vaccination in years	24 (0-55)

ITP= Idiopathic thrombocytopenic purpura

Sx= splenectomy

For gender and reason for asplenia, data are given in numbers of patients with percentages recorded in parentheses. For age at Sx, age at vaccination and time from Sx to vaccination, data are given in median number of years, with the range in parentheses

Table 2. *Neisseria meningitidis* serogroup C: response to vaccination

≥ 2.0 µg IgG/mL pre-vaccination: n (%)	14 (12)
≥ 2.0 µg IgG/mL post-vaccination: n (%)	78 (67)
Seroconversion: n (%)	93 (80)

Data are given in numbers of patients with percentages in parentheses

Seroconversion: a ≥ fourfold increase in antibody concentration after vaccination

Table 3. Characteristics of responders and hypo-responders to one dose of MenC conjugated vaccine

	responders	hypo-responders
Patients (n)	78	38
Male gender	39 (50)	20 (53)
Reason for Sx		
Trauma	31 (40)	4 (11)
Haematological malignancy	12 (15)	6 (16)
Other reasons	35 (45)	28 (74)
Age at Sx in years	27 (5-76)	30 (3-78)
Age at vaccination in years	52.0 (23-86)	53.5 (29-83)
Time from Sx to vaccination in years	25 (0-55)	23 (2-50)
Previously vaccinated	14 (18)	3 (8)

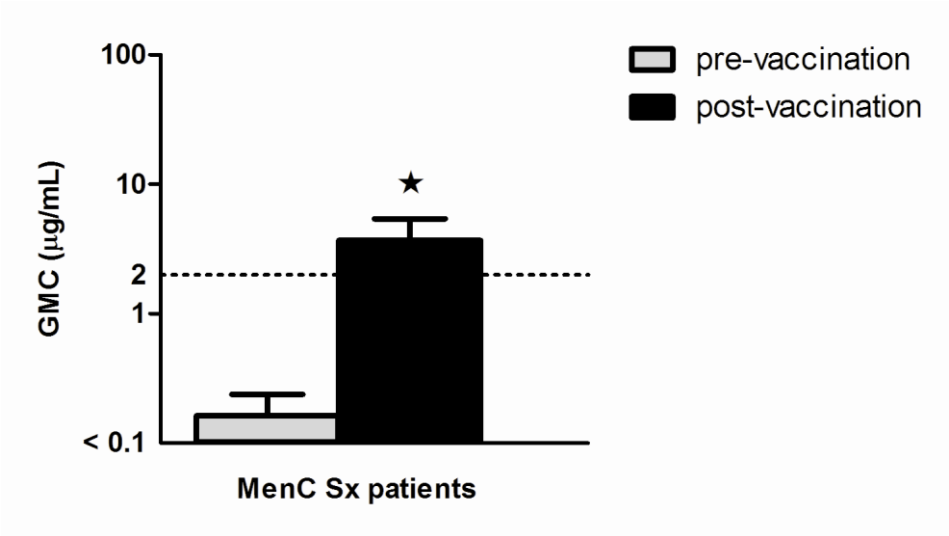
Sx= splenectomy

Hypo-responder = MenC-specific IgG < 2.0 µg IgG/mL post-vaccination

For gender, reason for Sx and previously vaccinated, data are given in numbers of patients with percentages recorded in parentheses. For age at Sx, age at vaccination and time from Sx to vaccination, data are given in mean number of years, with the range in parentheses



Figure 1. Geometric mean concentrations of MenC IgG before and after MenC conjugate vaccination in asplenic patients (n=116)

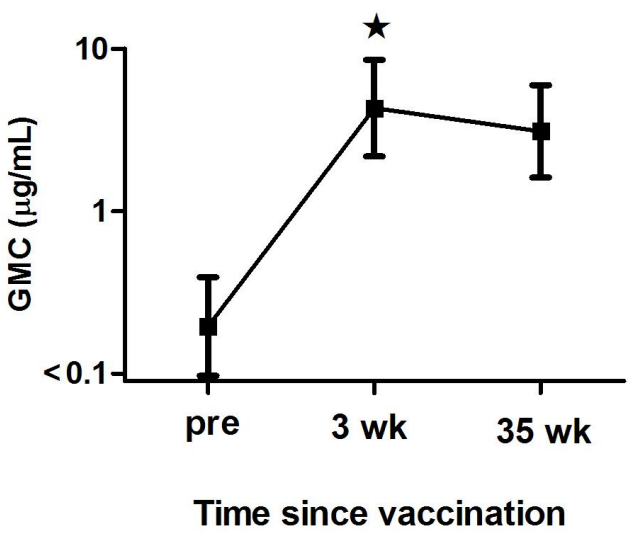


GMC = geometric mean concentration

Sx = splenectomy

★ statistically significant increase ( $p < 0.05$ ) in antibody concentration post-vaccination compared to pre-vaccination

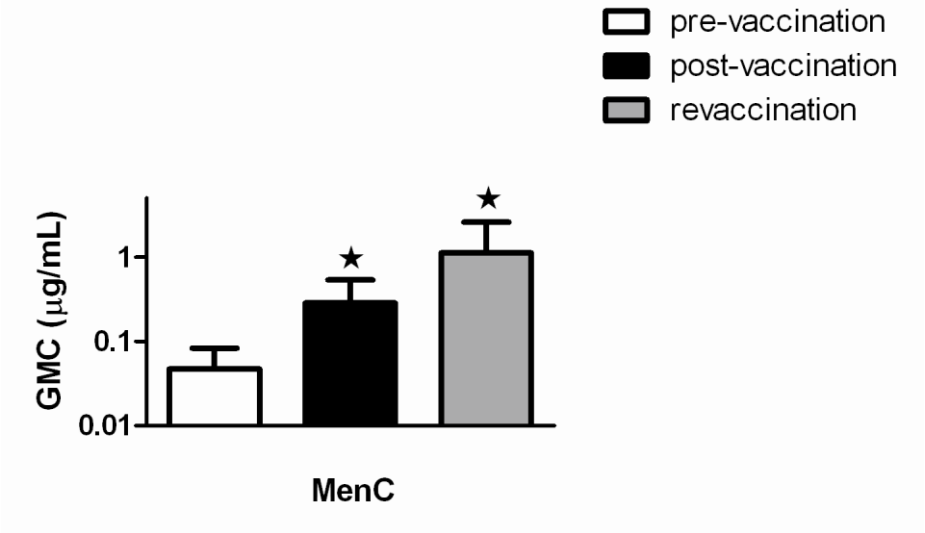
Figure 2. Geometric mean concentrations eight months after vaccination



GMC: Geometric mean concentration in µg/mL with 95% confidence interval

★ statistically significant increase ( $p < 0.05$ ) in antibody concentration post-vaccination compared to pre-vaccination

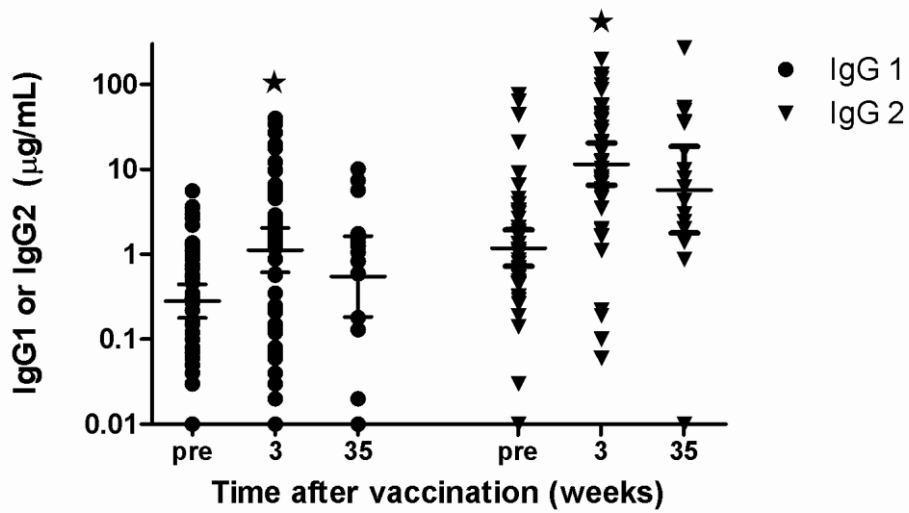
Figure 3. Geometric mean concentrations after MenC revaccination (n=22)



GMC: Geometric mean concentration in µg/mL with upper 95% confidence interval

★ statistically significant increase (p < 0.05) in antibody concentration post-vaccination compared to pre-vaccination

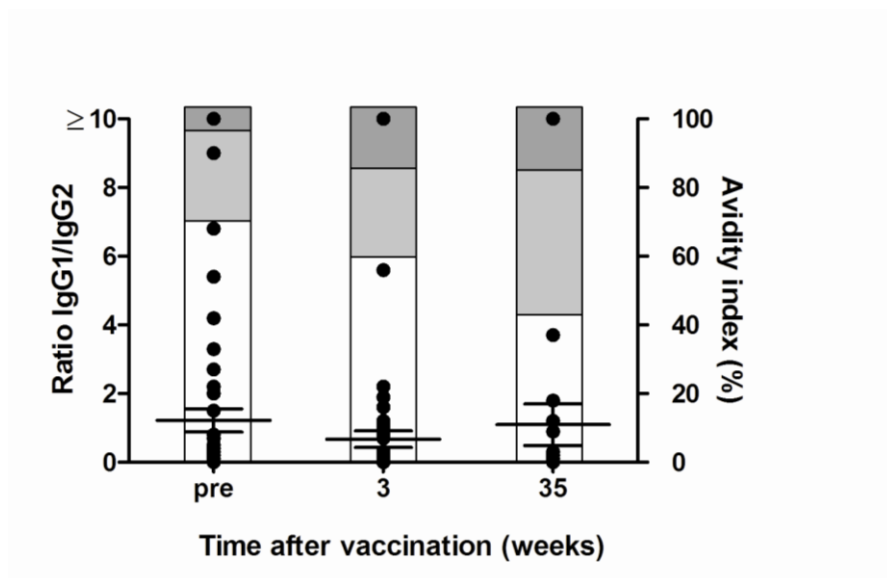
Figure 4a. Concentrations of MenC IgG1 and IgG2 in relation to time after vaccination



IgG with lines at mean with 95% confidence interval

★ p < 0.05

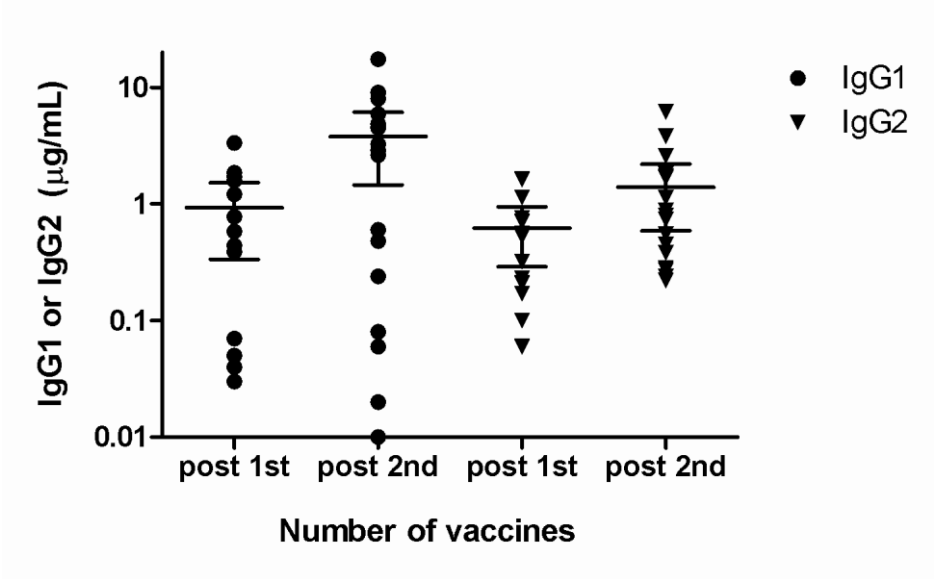
Figure 4b. Development of MenC IgG1/IgG2 ratio compared to changes in avidity



Dots: IgG with lines at mean with SEM

Columns: Avidity indices: dark gray 67-100%, light gray 34-66%, white 0-33%

Figure 4c. Concentrations of MenC IgG1 and IgG2 after the first and second conjugate vaccine in hypo-responders



IgG with lines at mean with 95% confidence interval