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Avidity of Serogroup A Meningococcal IgG Antibodies after Immunization with Different Doses of a Tetravalent A/C/Y/W135 Polysaccharide Vaccine

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Abstract

In the absence of an affordable conjugate meningococcal vaccine, mass vaccination campaigns with polysaccharide vaccines are the means to control meningitis epidemics in sub-Saharan Africa. Facing global vaccine shortage, the use of reduced doses, which have been shown to be protective by serum bactericidal activity, can save many lives. In this study, we investigated the antibody responses and avidity of IgG antibodies evoked against the serogroup A capsule of *Neisseria meningitidis* by different doses of an A/C/Y/W135 polysaccharide vaccine. Volunteers in Uganda were vaccinated with 1/10, 1/5 or a full dose (50 μg) and revaccinated with a full dose after 1 year. Specific IgG geometric mean concentrations and geometric mean avidity indices (GMAI) were determined by a modified enzyme-linked immunosorbent assay (ELISA) using thiocyanate as a chaotropic agent. After vaccination with 1/10 or 1/5 doses, the GMAI increased from 1 month to 1 year. One year following the initial dose, the GMAI levels were higher in the arm receiving reduced doses than for the arm receiving a full dose. Following the second full dose, avidity indices equalized at approximately the same level in the three arms. Although there are practical challenges to the use of reduced doses in the field, our findings suggest that reduced doses of polysaccharide vaccine are able to elicit antibodies of as good avidity against serogroup A polysaccharide as a full dose.

Introduction

Meningococcal disease continues to be a major public health problem in sub-Saharan Africa, in a region called the meningitis belt, stretching from Senegal in the west to Ethiopia in the east [1, 2]. This area is characterized by high seasonal endemicity and experiences recurring epidemics. The vast majority of cases of meningococcal disease in the area is caused by *Neisseria meningitidis* serogroup A, although epidemics caused by other serogroups like W135 and X also have been reported [3].

To prevent emerging epidemics from spreading, the World Health Organization (WHO) recommends the use of mass vaccination with polysaccharide vaccines in the population at risk when an epidemic threshold is reached [4]. However, protective immunity of the polysaccharide vaccines is short-lasting and considered sub-optimal in small children [5]. Access to affordable conjugate meningococcal vaccines in large quantities for Africa is not expected to be generally available before some time [6, 7]. There is ongoing, on the other hand, a very promising project on conjugated meningococcal vaccine against serogroup A, the Meningitis Vaccine Project. This is a partnership between the WHO and the Program for Appropriate Technology in Health [6]. Its goal is to eliminate epidemic meningitis as a public health problem in sub-Saharan Africa by developing and providing an affordable conjugated vaccine against serogroup A meningococci. The vaccine has been proven safe and was introduced in the worst affected African countries in December 2010 [8].

In the meantime, polysaccharide vaccines are still much needed. However, over the last years, the availability of polysaccharide vaccines has been uncertain [9]. If faced with vaccine shortage, the use of reduced doses of polysaccharide vaccines may save many lives. The current doses of licensed tetravalent polysaccharide vaccines contain 50 μg of each polysaccharide component. Studies in

young adults in the United States in the 1970s and 1980s showed that lower doses of polysaccharide were as effective as 50 μg in inducing serum bactericidal antibody levels that are assumed to be protective against meningococcal disease [10, 11]. We have previously shown that the same is true in African children and teenagers for serogroup A, Y and W135 [12], with 1/5 of the dose (10 μg) inducing similar levels of bactericidal antibodies as a full dose (50 μg). In this study, which aims to improve the understanding of the immunological mechanisms of reduced doses, only response to the serogroup A portion of the vaccine was investigated, as it is the serogroup causing the majority of disease in Africa.

To measure functional antibody activity and thereby the effect of meningococcal vaccines, serum bactericidal activity (SBA) have become the most widely accepted method to determine protection against disease [13], although the only correlate to clinical protection for serogroup A meningococci to date is based on results from an efficacy trial conducted in Finland in the 1970s. The investigators concluded the correlate of protection to be an anti-A polysaccharide immunoglobulin concentration of 2 μg/ml, as determined by radioimmunoassay [14]. Methods that only quantify IgG antibody levels have later shown poor correlation with functional antibody activity as measured by SBA [15–18]. Antibodies of high avidity, on the other hand, have shown to be more active in bacteriolysis and passive protection in animal models in studies of immune responses against *Haemophilus influenzae* type b, *Streptococcus pneumoniae* and serogroup C meningococci [19–23]. Antibody avidity can be defined as the total strength of the multivalent interactions between antibody and antigen. Methods to detect high-avidity antibodies have also been developed for meningococcal polysaccharides [24, 25]. The avidity of antibodies and proportions of high-avidity antibodies have shown higher correlation with SBA levels in sera obtained after vaccination with conjugated and unconjugated serogroup C or A polysaccharide vaccines [24–26].

Chaotropic agents, such as thiocyanate ions, have the ability to disrupt antibody–antigen bindings and could be used to determine the avidity of antibodies because tolerance to thiocyanate elution has been suggested to be proportional to the strength of the antigen–antibody interaction [27]. In the study presented here, we have used a modified chaotropic ELISA method [28, 29] to estimate the avidity of IgG antibodies against serogroup A polysaccharide elicited after immunization of children and teenagers in Uganda with full dose, 1/5 dose or 1/10 dose of a tetravalent polysaccharide vaccine.

Materials and methods

Study population and vaccine. The population included in this study was a subgroup of 115 individuals taking part in a larger vaccine trial study in Uganda with 750 volunteers aged 2–19 years [10]. The administered vaccine was Menomune® from Sanofi Pasteur, a tetravalent A/C/Y/W135 polysaccharide vaccine.

The trial participants were recruited on a voluntary basis, in proportions matching the age distribution extracted from the '2002/03 Uganda National Household Survey.' All volunteers were residents in a rural area of the Mbarara District, a location that had not experienced recent epidemics of meningococcal meningitis. The initial 750 participants were block randomized by age (2–4; 5–9; 10–14 and 15–19 years) into three different dosage arms: the first arm (n = 291) receiving a full dose (50 μg of each polysaccharide) of the tetravalent polysaccharide vaccine, the second arm (n = 225) receiving 1/5 dose (10 μg) and the third arm (n = 234) receiving 1/10 dose (5 μg).

The 115 individuals investigated here were randomly recruited from the larger study population to receive a second, full dose (50 μg) of tetravalent polysaccharide vaccine after 1 year. A full dose was chosen for revaccination because in case reduced doses will be used in an outbreak setting, subsequent vaccination in later outbreaks would most likely be with full doses of 50 μg. The subjects were representative for the larger study population in age, sex and doses. Initially, 120 individuals were recruited, but at the 1-year follow-up visit, post-initial dose, only 38 participants were present from the group receiving a full dose, 39 from the group receiving 1/5 dose and 38 from the group receiving 1/10 dose initially.

Serum samples and reference serum. Five serum samples were drawn from each individual at various visits: immediately before vaccination; 1 month after the first dose; 1 year after the first dose (immediately before receiving the second dose); 1 month after the second dose; and 1 year after the second dose. As a reference, we used the standard CDC 1992, anti-A and anti-C meningococcal polysaccharide serum (99/706), obtained from the National Institute for Biological Standards and Controls, Hertfordshire, UK, that was assigned a value of 91.8 μg/ml of IgG against serogroup A polysaccharide [30].

Measurement of antibody avidity. The concentration and avidity of IgG antibodies against serogroup A polysaccharides were measured using an ELISA method based on a modification of previously described assays [28, 29, 31]. In brief, Nunc Maxisorb 96-well microtitre plates were coated with serogroup A meningococcal polysaccharide in complex with methylated human serum albumin at a final concentration of 5 μg/ml of each component. Coated plates were incubated overnight and stored up to 14 days at +4 °C. The day of analysis, plates were washed in PBS, pH 7.0, with 0.1% Brij and 0.02% sodium azide, and incubated for 1 h at room temperature

with 3% foetal calf serum (FCS) in PBS with 0.02% sodium azide. Test sera were twofold diluted at concentrations 1:100 up to 1:800 with dilution buffer containing PBS with 3% FCS, 0.1% Brij and 0.02% sodium azide. Reference sera were diluted at concentrations 1:400 to 1:51,200. Each well was added 100 μl of test sera or reference sera, set up in duplicate. All five sera from each individual were tested on the same plate. The microtitre plates were then incubated overnight (16–20 h) at +4 °C. The following day, the plates were washed and half the plate was added a 120-mM dilution of ammonium thiocyanate in PBS, while the other half and the reference serum was added only PBS for incubation at room temperature for 30 min. To determine the optimal assay conditions for measuring the avidity, different concentrations of ammonium thiocyanate (ranging from 0 to 1.0 M) were initially tested with a representative subset of serum samples from Ugandan vaccinated with the polysaccharide vaccine. The use of a 120-mM ammonium thiocyanate solution resulted in strong reduction in ELISA optical densities (OD) values for most of the sera, whereas other serum samples were less affected. Thus, a 120-mM solution was considered the best concentration to discriminate between high- and low-avidity sera. After incubation with the chaotropic agent and washing, secondary antibody [goat anti-human IgG (γ-chain specific)-alkaline phosphatase antibody; Sigma-Aldrich, St. Louis, MO, USA] was added and the plates were further incubated at +37 °C for 2 h. After being washed, the plates were developed using 1 mg/ml p-nitrophenyl phosphatase substrate prepared in 10% diethanolamine buffer. OD values at 405 nm were read when the reference serum at dilution 1:400 had reached an OD of approximately 2. OD values for wells incubated without sera were subtracted as background.

IgG antibody concentrations were calculated using a 4-parameter logistic curve-fitting analysis [32] against the standard reference curve. Multiple data points obtained from dilutions that yielded OD values in the linear portion of the curve were averaged, and IgG geometric mean concentrations (GMC) were calculated from wells where no thiocyanate was added. Avidity indices (AI) and geometric mean AI (GMAI) were calculated as percentage of antibodies that remained bound after treatment with thiocyanate, as described by Antilla *et al.* [28] and Romero-Steiner *et al.* [29].

SBA. Serum bactericidal activity against serogroup A strain F8238 (4/21:P1.20,9), using baby rabbit serum as complement (PelFreez Biologicals, Brown Deer, WI, USA), of sera obtained before and 1 month after the first vaccination has been tested using a method described by Maslanka *et al.* [18]. SBA titres were defined as the reciprocal of the serum dilution with ≥50% killing of the initial inoculum. The results have been published by Guerin *et al.* [12].

Statistical analyses. Geometric mean concentrations and GMAI with 95% confidence intervals were calculated for each group at all time points. Unpaired *t*-tests were used to compare means between different groups, and paired *t*-tests were used to compare different time points within one group. For correlation analyses, we used Pearson correlation test. Significance level was set at a 5% level. Data were analysed using GRAPHPAD Prism version 4.02.

Ethical considerations. Written informed consent in the local language was obtained from the parents or guardians of every volunteer under 18 years of age or by the volunteers themselves if older than 18 years. The study was approved by the Faculty Research and Ethics Committee of the Mbarara University of Science and Technology (MUST), the MUST Institutional Review Board, the Uganda National Committee of Science and Technology and the Regional Committee for Medical Research Ethics in Norway. The trial was registered at Clinicaltrials.gov (NCT00271479).

Results

Table 1 shows the anti-serogroup A-specific IgG concentrations in the different dose groups at five sampling points. We were not able to determine IgG concentrations <0.5 μg/ml; thus, we assigned a value of 50% of the lowest determined value (0.25 μg/ml) as IgG concentration of sera <0.5 μg/ml to calculate the GMCs. Before vaccination, there was no significant difference in anti-A polysaccharide GMC between the three different dose

Table 1 Geometric mean concentrations (GMC) in μg/ml of specific IgG against serogroup A polysaccharide after vaccination with different doses of A/C/Y/W135 polysaccharide vaccine initially and followed by a full second dose after 1 year.

Dose (sample size)	1/10 dose (N = 34)		1/5 dose (N = 37)		Full dose (50 μg) (N = 37)	
Sampling	GMC	95% CI	GMC	95% CI	GMC	95% CI
Before vaccination	1.0	(0.7–1.7)	1.2	(0.8–1.7)	0.9	(0.6–1.2)
1 month after 1st dose	4.5	(3.1–6.6)	6.2	(4.3–9.1)	13.8	(9.9–21.3)
1 year after 1st dose	2.5	(1.8–3.5)	3.6	(2.5–5.1)	6.7	(4.4–10.1)
1 month after 2nd dose	18.9	(14.2–25.2)	19.5	(14.2–26.9)	17.4	(11.5–26.2)
1 year after 2nd dose	9.1	(7.1–11.6)	9.6	(7.0–13.2)	8.5	(5.8–12.6)

Table 2 Geometric mean avidity indices[a] (GMAI) of IgG antibodies against serogroup A polysaccharide after vaccination with different doses of A/C/Y/W135 polysaccharide vaccine initially and followed by a full second dose after 1 year.

Dose (sample size)	1/10 dose (N = 23)		1/5 dose (N = 27)		Full dose (50 μg) (N = 31)	
Sampling	GMAI	95% CI	GMAI	95% CI	GMAI	95% CI
Before vaccination	ND[b]		ND[b]		ND[b]	
1 month after 1st dose	43.3	(36.5–51.4)	44.6	(38.4–51.8)	37.9	(32.6–44.2)
1 year after 1st dose	54.4	(48.0–61.7)	53.9	(45.6–63.6)	40.7	(34.9–47.5)
1 month after 2nd dose	41.7	(35.6–48.9)	41.5	(37.5–46.0)	39.5	(34.5–45.3)
1 year after 2nd dose	38.4	(32.9–44.8)	41.4	(37.6–45.5)	35.3	(30.6–40.8)

[a]Calculated as percentage of IgG antibodies still bound after adding 120 mM thiocyanate.
[b]ND, Not done. Because of low IgG antibody levels before vaccination, avidity indices could only be determined for a minority of the sera. Calculation and GMAI were therefore omitted.

groups. One month after the first vaccination, the IgG GMCs increased significantly in all three groups (P = 0.01, P < 0.01, P < 0.0001 for 1/10, 1/5 and full doses, respectively), with the greatest increase in the individuals receiving a full dose. One year following vaccination, the IgG levels had declined by 40–50% compared to 11 months earlier in all groups, although they were still significantly higher than before vaccination (P < 0.0001, P = 0.02, P < 0.0001 for 1/10, 1/5 and full doses, respectively). The IgG concentration in the full-dose group was still significantly higher than in the 1/10 dose (P = 0.0002), but not statistically significantly higher than in the 1/5 dose. Following vaccination with a second, full dose for all individuals, we found that those who initially had received reduced doses had IgG responses at the same level as those receiving a full dose. One month after the second dose, the IgG levels were significantly higher for the 1/10 (P < 0.0001) and 1/5 dose (P = 0.0012) as compared to the levels observed 1 month after the first dose, whereas there was no difference in the full-dose group.

With a threshold of 2 μg/ml anti-A polysaccharide antibodies that was estimated to be protective in the trial in Finland [14, 33], we found that the number of individuals presumably protected increased over the first month after the first dose: from 32% before vaccination to 88% after receiving a 1/10 dose, from 38% to 92% after receiving a 1/5 dose and from 27% to 92% in the full-dose group. One year later, the percentage of individuals with IgG concentrations still above this level had declined to 65%, 78% and 78%, respectively.

We were not able to determine AI with sufficient accuracy in sera from individuals with IgG concentrations <1.2 μg/ml. Therefore, in sera collected before vaccination, AI were not determined. Following vaccination, however, we were able to measure AI in 70% of the individuals (61%, 69% and 82% of the groups that received 1/10, 1/5 and full dose, respectively). GMAI following the first and second immunization are shown in Table 2. No significant differences in avidity between the different dose groups were found 1 month after the first dose. One year after vaccination, we found a significant increase in the avidity in the groups receiving reduced doses of 1/10 or 1/5 as compared to the avidity levels observed 1 month after vaccination (P < 0.01 for both groups) as well as compared to the full-dose group (P < 0.01). After revaccination with a full dose, the AI decreased in all groups and fell to levels equal to those seen in the full-dose group 1 month after the first full dose and remained at the same level over the course of the second year in all dose groups.

There were no significant differences in IgG concentration and avidity in the different age groups within and between dose arms (data not shown).

Figure 1A shows SBA titres against serogroup A using the baby rabbit complement plotted versus IgG quantities against A polysaccharide from sera collected 1 month after the first vaccination for the three dose groups. Using Pearson correlation test, we found a significant correlation between SBA titres and IgG concentrations 1 month after the first vaccination only in the group receiving the full dose (r = 0.50, P = 0.002). Using the IgG quantity after adding 120 mM thiocyanate, a similar correlation was found (r = 0.44, P = 0.012) also only for the full-dose group (Fig. 1B).

Discussion

Avidity determinations have been used as a surrogate marker for protection in several studies, theoretically suggesting that despite low quantities of antibodies, individuals can be protected from infection if they are primed for memory response [24–26, 28, 29, 34]. In this study, we investigated the avidity index and quantity of IgG antibodies evoked against serogroup A polysaccharide by different doses of a tetravalent meningococcal polysaccharide vaccine. We used a modified ELISA method with thiocyanate as a chaotropic agent [28, 29].

There are several ways to measure avidity, but solid-phase assays like ELISA are among the most common, because they are simple and reproducible methods that do not require large amounts of antibodies. On the other

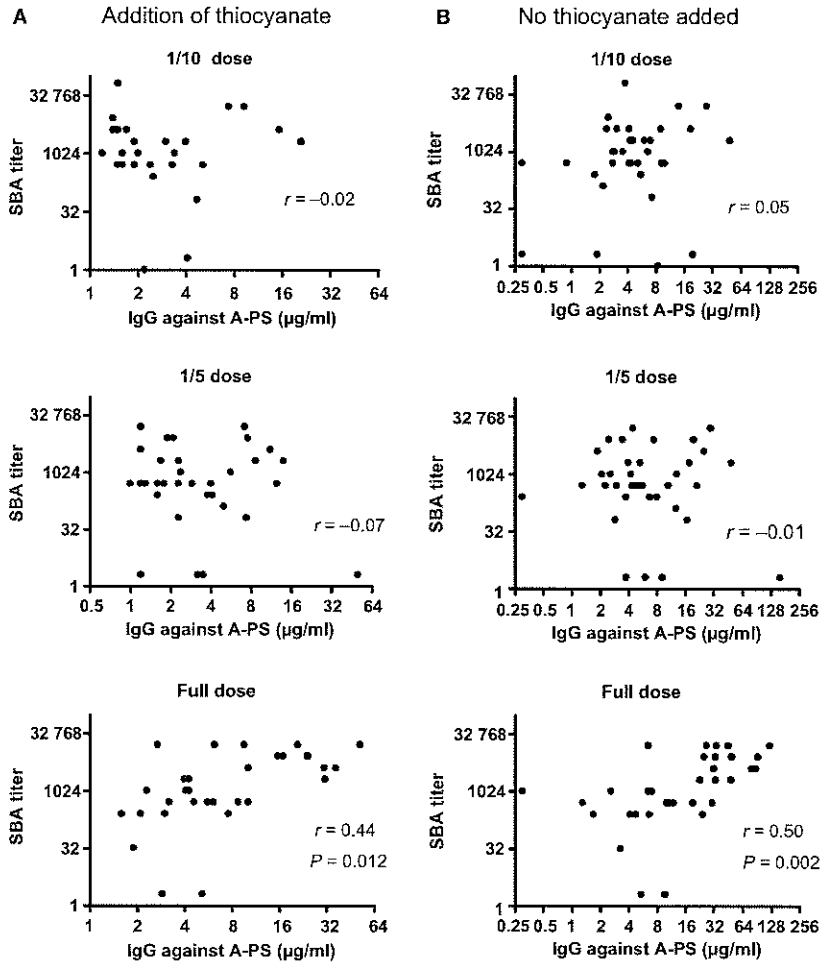

Figure 1 Correlation between serum bactericidal activity (SBA) and serum IgG antibodies against meningococcal serogroup A polysaccharide 1 month after vaccination with A/C/Y/W135 polysaccharide vaccine with the addition of 120 mM ammonium thiocyanate as chaotropic agent (A) and without (B). Pearson correlation coefficients are shown.

hand, there are several methodological weaknesses concerning all ELISA methods using a chaotropic agent to measure avidity. As pointed out by Harris *et al.* [35], these methods do not solely measure avidity; they also measure the antibodies' stability to the chaotropic agent. Thiocyanate may influence on antibody–antigen binding by disrupting various non-covalent interactions such as hydrogen bonds, electrostatic bindings, van der Waals forces and hydrophobic interactions [36]. These interactions also contribute in maintaining antibody conformation, which therefore may be altered as well. When performing an assay on a solid phase, interactions between adjacent antibodies, for instance their Fc regions, might also influence the results, as well as antigen and antibody density and steric hindrances for binding [36].

With numerous different methods for avidity measurement, it is difficult to compare results from different studies, but the unifying principle is that higher concentrations of the chaotropic agent are required to disrupt higher-avidity antigen–antibody bindings. Romero-Steiner *et al.* [29] compared three different ELISA methods measuring antibody avidity against *H. influenzae* type b polysaccharide and concluded that a method using serial dilution of serum and a single dilution of thiocyanate added after antigen–antibody binding, as we have used here, was to be preferred.

The findings that the AI in the study arms receiving reduced doses increased over the first year and were higher than for the arm receiving full dose at 1 year after the first vaccination suggest that lower dose of serogroup A meningococcal polysaccharide may lead to antibody maturation. In general, polysaccharides, like the meningococcal serogroup C polysaccharide, elicit immune responses in a classical T cell-independent way [25, 37–39]. The increase in avidity following the initial vaccination with reduced doses and the fact that the avidity did not decrease significantly during the second year for any of the doses confirm the unusual behaviour of serogroup A polysaccharides, as has already been pointed out by others [10, 25]. The increasing avidity in the reduced dose arms also renders the possibility that lower doses might be better in promoting antibody of high affinity. This has previously been shown to be the case in experimental studies in mice, where lower doses of antigen lead to greater increases in

avidity [40]. This could be attributable to a competition for antigen between antigen-specific B cells with receptors of varying affinity when antigen is scarce, favouring the production of high-avidity antibodies. The use of a limited amount of antigen in polysaccharide vaccines could be of special importance as the T cell-independent nature of polysaccharides prevents affinity maturation. Interestingly, a similar increase in IgG antibody avidity with lower doses of antigen was also found by Romero-Steiner and co-workers [41] in a study with reduced doses of *H. influenzae* type b conjugate vaccine.

The levels of anti-A polysaccharide IgG were found to increase in a dose-dependent manner following the first vaccination. However, when using the threshold of 2 μg/ml total IgG [14, 33], more than 80% in all dose groups were apparently protected. The relevance of this threshold is debatable, especially in an African population. The amounts of IgG found before vaccination in this study were similar to those found by others in unvaccinated children and adolescents from Saudi Arabia and Uganda [42, 43].

We did not find any significant differences in IgG responses according to the age of the participants. In contrast, Al-Mazrou *et al.* [42] showed age-dependent increases in IgG responses in children under 5 years of age. However, in our study, no children under the age of 2 years were included, and there was a low number of individuals in each age group.

We observed no decrease in neither avidity nor IgG levels after revaccination. Thus, this study renders no evidence of induction of hyporesponsiveness to serogroup A polysaccharide, which is in agreement with the results of other investigators [44]. However, as we investigated response only to the serogroup A portion of the vaccine, hyporesponsiveness to the other serogroups, especially serogroup C, for which there has been repeated evidence of hyporesponsiveness [45, 46], cannot be excluded.

A better correlation between avidity and SBA activity is believed to be one advantage of avidity determinations, as opposed to measuring only the concentrations of IgG antibodies [22–24, 26]. As shown in Fig. 1, a significant correlation between the SBA results and IgG concentrations was observed in the assay both with and without thiocyanate only in the groups receiving a full dose; for the reduced doses, no correlation was found and the correlation was not improved by adding the chaotropic agent. This is in contrast to the findings of Granoff and colleagues with meningococcal serogroup C polysaccharide who found a better correlation with the use of thiocyanate using another methodology for the avidity assay [24].

Concerning the SBA method, significant differences in antibody titres have been reported when comparing SBA titres with baby rabbit or human serum as complement sources [18, 24, 47]. Baby rabbit complement is the most widely used complement source for measuring SBA against serogroup A, but the use of human complement might be more relevant in mimicking the *in vitro* situation.

To further investigate this relationship between avidity and functional antibodies, all sera were tested with an in-house serogroup A SBA using human sera as the source of complement. While this method yielded good responses and high titres when testing Norwegian adults vaccinated with a serogroup A-containing vaccine, only eight of the 115 individuals included in this study had a titre ≥4 after the first dose of vaccine. Therefore, we found no correlation between avidity measurements and SBA with human complement, and there were no significant differences between the different dose groups.

The lack of correlation observed here for serogroup A with reduced doses, using SBA with both rabbit and human sera as complement sources, might be attributable to several factors. There might be limitations in the methods used, both detecting avidity indices and functional bactericidal activity. The lack of correlation may also be explained by an undetectable increase in avidity following vaccination with unconjugated polysaccharide vaccines, which in comparison with conjugated vaccines has been shown to be limited [25].

Another factor that might be important is the fact that we have analysed only total anti-A polysaccharide IgG concentrations, not taking Ig isotypes and subclasses into consideration, which are suggested to be of significance, as IgM antibodies can be bactericidal and some IgG subclasses play a greater role in eliciting bactericidal responses than others [43, 48–50].

This study shows that reduced doses of meningococcal polysaccharide vaccine are able to elicit antibodies with as good avidity as a full dose. Together with the SBA findings previously published [12], this provides immunological indication for the use of reduced doses. There are, however, numerous practical challenges to consider before one can recommend use of reduced doses in the field. The practical feasibility of splitting the dose vials, the need for different sized syringes, etc. have first to be overcome. In a setting of severe vaccine shortage during an epidemic, WHO do recommend that the use of reduced doses should be considered [51].

Finally, further studies are needed to determine the importance of antibody avidity on functional activity of anti-A polysaccharide IgG antibodies and its role in clinical protection against serogroup A meningococcal disease. Similar studies encompassing all four serogroups would also be valuable.

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Conflict of interest

The authors declare no financial or commercial conflict of interest.

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