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Antibodies against *Neisseria meningitidis* serogroups A, C, W and Y in serum and saliva of Norwegian adolescents

Sara Viksmoen Watle [a,b,*], Bente Børud [a], Ida Laake [a], Marta Baranowska-Hustad [a], Diane Bryant-Bratlie [a], Terese Bekkevold [a], Dominique A. Caugant [a,b], Gro Tunheim [a,1], Lisbeth Meyer Næss [a,1]

[a] Division of Infection Control, Norwegian Institute of Public Health, P.O. Box 222, Skøyen, 0213 Oslo, Norway
[b] Institute of Health and Society, Faculty of Medicine, University of Oslo, P.O. Box 1078, Blindern, 0316 Oslo, Norway

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ABSTRACT

Introduction: The incidence of invasive meningococcal disease (IMD) among Norwegian 16–19-year-olds was 1–7/100,000 in the decade before the COVID-19 pandemic, with serogroup Y (MenY) dominance. In contrast to many other European countries, meningococcal vaccines are not part of the national immunisation program (NIP) in Norway. This cross-sectional study aimed to measure the degree of natural immunity against *Neisseria meningitidis* among adolescents in Norway to evaluate the need for introducing tetravalent meningococcal conjugate vaccine (MCV4) in the NIP.

Materials and methods: Serum and saliva samples were collected from students in upper and lower secondary schools in Norway in 2018. Samples were analysed for meningococcal capsular polysaccharide (PS)-specific antibodies using a bead-based multiplex immunoassay. PS-specific antibody levels were linked to data on meningococcal carriage, vaccination status and risk factors for carriage (assessed with questionnaire) and analysed by linear regression of log transformed concentrations. A subset of samples from unvaccinated individuals was analysed for serum bactericidal antibodies (SBA).

Results: A total of 1344 participants, median age 16 years (range 12–24), were included in the study. Overall, 60.9% of the participants were female and 1137 (84.6%) were not vaccinated with MCV4. PS-specific antibody concentrations in serum and saliva were low among unvaccinated individuals for all serogroups and only 6.7–20.0% of the subpopulation with high PS-specific antibodies assessed with SBA had protective levels. Unvaccinated MenY carriers had higher levels of MenY anti-PS IgG in serum and IgA in saliva than those not carrying MenY. Use of Swedish snus was associated with lower anti-PS IgG levels in serum and waterpipe use with lower anti-PS IgG levels in saliva.

Conclusion: Unvaccinated adolescents in Norway have a low degree of natural immunity against the serogroups of *N. meningitidis* predominating among cases of IMD in this age group. Therefore, introduction of MCV4 for adolescents in the NIP is recommended.

1. Introduction

Neisseria meningitidis, the meningococcus, is a human commensal colonising the upper respiratory tract, a condition referred to as carriage. Meningococcal carriage may, in rare instances, lead to the development of invasive meningococcal disease (IMD), with high morbidity and mortality [1–2]. The bacterium is classified into serogroups based on the structure of the polysaccharide (PS) capsule, its most important virulence factor [3]. Among the 12 known serogroups, six (A, B, C, W, X and Y) are responsible for most invasive cases. Adolescents and young adults have increased risk of IMD, due to higher carriage rates and behaviour-related higher risk of transmission [4–7].

In the decade before the COVID-19 pandemic, the annual incidence of IMD in the general population in Norway was 0.3–0.8 per 100,000, whereas the incidence among 16–19-year-olds was 1–7 per 100,000 in the same period [8]. Serogroup B and Y have dominated in the general

* Corresponding author at: Norwegian Institute of Public Health, P.O. Box 222, Skøyen, 0213 Oslo, Norway.
E-mail address: SaraSofieViksmoen.Watle@fhi.no (S.V. Watle).
[1] Both contributed equally.

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population. However, almost all cases among adolescents were caused by serogroup Y. In 2018–19, the carriage rate among 18-year-olds in Norway was 16.4%, with a dominance of serogroup Y among encapsulated isolates [9]. IMD among adolescents in Norway have been linked to an extensive celebration of graduation from secondary school among 18–19-year-olds, the *russ celebration* [10]. The tetravalent ACWY meningococcal conjugate vaccine (MCV4) has been recommended for the 17–19-year-olds since 2011 and 16–19-year-olds since 2012. The vaccine is not included in the national immunisation program (NIP) and is therefore not funded by the government. Around 60% of 18-year-olds follow the recommendation [11].

Protective antibodies against meningococci are induced by vaccination but can also be induced by carriage of the bacterium, or through cross-protective antibodies derived from carriage of antigenically similar bacteria such as *Neisseria lactamica* or *Escherichia coli* [12–16]. Whereas antibodies in serum are crucial for the protection against IMD [17–18], salivary antibodies may contribute to protection against acquisition and carriage of *N. meningitidis* [19]. As IMD can develop into a fatal outcome within hours, these circulating antibodies need to be present in sufficient amount at the onset of infection as recall responses of memory cells will not occur in time to control the infection [20].

Analysis of serum bactericidal antibodies (SBA) is considered the correlate of protection against IMD and is the basis for licensure of meningococcal vaccines [21–23]. Even though they are not the established gold-standards, immunoassays analysing meningococcal PS-specific antibodies are easier to perform than SBA assays and can be used in immunosurveillance studies [24–26]. Anti-PS immunoglobulin (Ig) G levels in serum of vaccinated children have been shown to correlate with protection against serogroup A IMD [27].

The aim of this study was to measure the level of meningococcal PS-specific antibodies in serum and saliva among adolescents in Norway according to age, meningococcal carriage, vaccination history and risk factors for meningococcal carriage. We were particularly interested in characterising the immunity in unvaccinated participants in order to assess the need for introducing MCV4 in the NIP for adolescents in Norway.

2. Materials and methods

2.1. Study design and study population

Students in lower (grades 8–10, ages 12–15) and upper (grades 11–14, ages 15–24) secondary schools in South-Eastern Norway were recruited to a cross-sectional study, as described previously [9]. In the current study, only the participants recruited from October-November 2018 were included as serum and saliva samples were only collected at that time. Informed consent was obtained from students aged $\geq$ 16 years, while parental consent was requested for younger students. The study was approved by the Regional Committee for Medical and Health Research Ethics, South-East Norway (reference number 2018/465). In total, 1367 individuals from 12 lower and 13 upper secondary schools consented to participate in the study.

2.2. Data collection

Serum samples were collected by venepuncture into tubes without anticoagulant (Vacutainer serum tubes, BD, Franklin Lanes, NJ, USA) and transported to the laboratory in styrofoam containers at room temperature. Saliva samples were collected using Salivette® Cortisol (Sarstedt, Nümbrecht, Germany). The absorbent synthetic swab was placed in the mouth for 90s, transferred to a storage tube without preservatives, and immediately placed in racks in styrofoam containers with ice. All samples reached the laboratory within 6 h of collection. At the laboratory, serum and saliva samples were centrifuged and aliquoted into Nunc cryotubes (Thermo Fisher Scientific Inc., Waltham, MA, USA). Serum samples were stored at −20 °C and saliva samples at −80 °C.

Presence of meningococcal carriage and degree of exposure to risk factors for meningococcal carriage were assessed from all participants through throat swabs and electronic questionnaire data from all participants, respectively, as previously described [9]. Vaccination status for MCV4 was obtained through linkage with the National Immunisation Registry SYSVAK, using the unique personal identification number assigned to all residents of Norway.

2.3. Study samples

Among the 1367 individuals consenting to participate, students without information on vaccination status or those missing results for both serum and saliva analyses were excluded from the study (11 and 12 individuals, respectively) (Fig. 1). Among the 1344 remaining participants included in the study, serum samples from 1313 participants (1109 unvaccinated) and saliva samples from 1260 participants (1054 unvaccinated) were analysed. After excluding individuals with incomplete questionnaire data, 1068 unvaccinated individuals with serum samples and 1018 unvaccinated individuals with saliva samples were included in the analyses of risk factors.

2.4. Multiplex immunoassay

Analyses of meningococcal anti-PS IgG in serum and anti-PS IgA and IgG in saliva against serogroup A (MenA), C (MenC), W (MenW) and Y (MenY) were performed using an in-house bead-based multiplex immunoassay (MIA) previously developed at Norwegian Institute of Public Health (NIPH) [28]. The assay is based on Luminex xMAP technology using the Bio-Plex 200^{TM} system with Bio-Plex Manager version 6.2 software (Bio-Rad, Hertfordshire, UK). Anti-meningococcal human reference serum CDC1992 99/706 (National Institute of Biological Standards and Controls (NIBSC), Hertfordshire, UK) was used as standard. The following modifications were adapted in the assay: 1) the purified capsular polysaccharides from MenA used was 98/722 and 13/262 and from MenW 01/428 and 21/180 (NIBSC, Hertfordshire, UK), 2) all incubations were run at 850 rpm, 3) R-phycoerythrin (PE) conjugated goat anti-human IgA (Jackson ImmunoResearch Europe Ltd., Ely, UK) was used for detection of IgA antibodies. Anti-PS serum IgG antibody levels were reported in μg/mL and anti-PS saliva IgA and IgG antibody levels in ng/mL and represent absolute concentrations [29].

2.5. Serum bactericidal antibody assay

SBA analyses were performed on a subset of serum samples from 30 participants. The participants were randomly selected based on the following criteria: age 15–16 years (considered as the possible age groups targeted for MCV4 vaccination in the national immunisation program), no registry in SYSVAK of MCV4 vaccination in the past 5 years, and *N. meningitidis* not detected by pharyngeal swabbing (non-carrier). Furthermore, the samples with anti-PS IgG against MenA, MenC, MenW and MenY in the higher range between the 50th and the 90th percentiles as measured by MIA were chosen for SBA analyses since the bactericidal activity induced by sera from unvaccinated individuals was in any case assumed to be relatively low.

A standardised SBA assay using baby rabbit complement (rSBA) was used with four target strains: MenA: F8238 (4/21:P1.20,9); MenC: C11 (16:P1.7–1,1); MenW: M01240070 (NT:P1.18–1,3); and MenY: M00242975 (2a:P1.5,2) [30–31]. Bacterial suspensions at concentrations of around 7×10^4 colony-forming units (CFU)/mL and baby rabbit complement (Pel Freez Biologicals, Milwaukee, WI, USA) was used. The reference serum CDC 1992 (NIBSC) was added to the complement source as positive control for MenA, MenC, MenW, and MenY.

Heat-inactivated test sera were diluted two-fold (starting at 1:4) in microtiter plates. The samples were incubated with bacteria and complement (active or heat inactivated) at 37 °C for 60 min before plating onto agar plates (Colombia agar with sheep blood, Thermo Scientific,

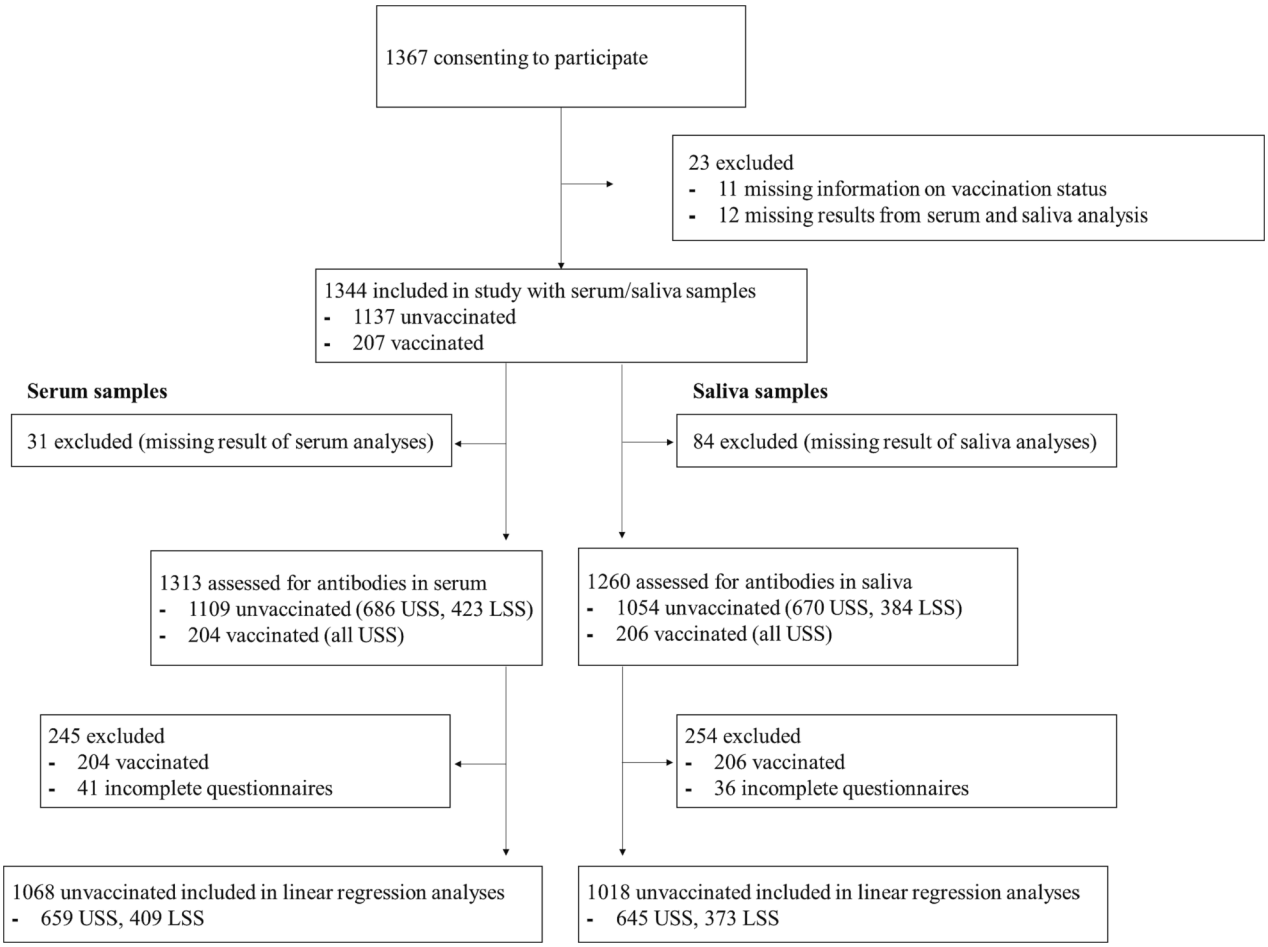

Fig. 1. Flow-chart for inclusion of participants in the study with regards to sampling, consent for assessment of meningococcal vaccination status and completion of questionnaires. Number of participants with serum and saliva sample results are shown. USS = upper secondary school students; LSS = lower secondary school students.

PB5039A, Vienna, Austria). CFU were counted (Sorcerer colony counter, Perceptive Instruments, England) after incubation of agar plates overnight at 37 °C and 5% CO_2. rSBA titres were expressed as the reciprocal of the highest serum dilution resulting in $\geq$ 50% killing of the meningococcal strain in comparison to a control without serum. The lower limit of quantitation was an rSBA titre of 4. Sera with rSBA titres $<$ 4 were assigned a titre of 2 for computational purposes.

2.6. Statistical analysis

Geometric mean concentrations (GMCs) in serum and saliva and proportions of participants with antibody levels $\geq$ 2 μg/mL in serum, i.e. assumed protective level [27], were calculated for MenA, MenC, MenW and MenY. We used cluster-robust standard errors to calculate 95% confidence intervals (CIs) for the proportions and means to account for possible dependencies within schools. Means were evaluated by vaccination status, age, sex, and carriage of *N. meningitidis*. Relation to carriage was only analysed for MenY as too few participants were carriers of the other serogroups. Age was defined as age in years at the time of sampling. When comparing unvaccinated and vaccinated individuals, we only included upper secondary school students, as all vaccinated individuals attended upper secondary schools. Moreover, we calculated pairwise Pearson correlation coefficients between log-transformed values of antibody levels in serum and saliva.

Furthermore, we assessed whether MenY PS-specific antibody levels in serum and saliva were associated with meningococcal carriage and risk factors (questionnaire data). Only unvaccinated individuals were included in these analyses. Quantile-quantile (q-q) plots of natural log-transformed values did not show any severe deviations from a normal distribution. We therefore used multivariable linear regression with the log-transformed values as the outcome. The generalised estimating equations approach [32] was used to fit the linear regression model. An exchangeable correlation structure was assumed. We used cluster-robust estimates of the standard errors. The model included smoking habits (use of cigarettes, e-cigarettes and waterpipe), exposure to passive cigarette smoking at home, use of Swedish snus (smokeless tobacco), attendance of youth gatherings and parties, and recent throat infection. For the variables assessing smoking habits and the use of Swedish snus, the response categories 'daily' and 'occasionally' were merged. In addition, we adjusted for sex, age, and carriage of *N. meningitidis* (MenY carriage and non-MenY carriage). Multivariable analysis on the association between MenY anti-PS IgG levels and risk factors was based on complete questionnaire data from 1068 individuals with serum samples and 1018 with saliva samples.

The analyses were performed using Stata SE 17.0 (Stata-Corp, College Station, Texas, USA). GraphPad Prism version 9 was used to generate figures (GraphPad Software, La Jolla, California USA).

3. Results

3.1. Characteristics of participants

A total of 1344 participants were included in the study (Fig. 1). Median age was 16 years (range 12–24 years). Over two-thirds (67.9%)

of the participants attended upper secondary schools (grades 11–14) and 93.4% of them were 16–18 years (range 15–24 years). In lower secondary schools (grades 8–10), the majority of students (97.5%) were 13–15 years (range 12–15 years). Overall, 60.9% of participants were registered as female (50.2% females in lower secondary schools and 66.1% females in upper secondary schools). Most of the participants were unvaccinated (84.6%). All vaccinated participants (n = 207) attended upper secondary schools in agreement with the national MCV4 recommendation for 16–19-year-olds. The median time since vaccination was 349 days (interquartile range 219–358 days). Carriage of *N. meningitidis* was detected in 102 (7.6%) of the 1344 participants. Most carriage isolates were capsule null (lacking the capsule operon, 46.1%) followed by genogroup Y (21.6%), non-groupable (isolates with deletions or stop codons in the capsule locus, 11.8%), genogroup B (11.8%), genogroup C (2.9%), genogroup W (2.9%) and genogroup X (2.9%). Of the 102 carriers, 20 were vaccinated with MCV4, corresponding to the proportion of vaccinated individuals in the study.

3.2. PS-specific antibodies in serum

Anti-meningococcal PS IgG GMCs in serum of the 1109 unvaccinated individuals per age, sex, and carriage of MenY are shown in Table 1. There were no major differences in anti-PS IgG GMCs in relation to age or sex, but the anti-PS MenY IgG GMCs were higher among the 18 carriers of MenY compared to the non-carriers. Furthermore, 66.7% (95% CI 40.4–85.5%) of the MenY carriers had anti-PS MenY IgG levels ≥ 2 μg/mL, compared to 23.9% (95% CI 20.9–27.1%) of those not carrying MenY.

Among the 890 participants in upper secondary schools from whom serum samples were analysed, the anti-PS IgG GMCs were four to 17 times higher among the vaccinated (n = 204) compared to the unvaccinated (n = 686) individuals for serogroups A, C, W and Y (Table 2). The proportion of the unvaccinated upper secondary school students with anti-PS IgG levels above 2 μg/mL was 43.7% (95% CI 38.8–48.8%) for MenA, 7.3% (95% CI 4.9–10.7%) for MenC, 14.1% (95% CI 10.8–18.3%) for MenW and 23.0% (95% CI 20.0–26.3%) for MenY, whereas for the vaccinated students, the proportions were 89.7% (95% CI 79.9–95.0%) for MenA, 67.6% (95% CI 60.0–74.5%) for MenC, 56.4% (95% CI 38.1–73.0) for MenW and 80.9% (95% CI 63.5–91.1%) for MenY.

3.3. Serum bactericidal antibodies

In the subset of samples analysed for SBA (n = 30), rSBA titres were obtained for all 30 samples for MenA, MenC and MenW, and for 12 samples for MenY. The remaining serum samples did not have defined killing curves for MenY. For the rSBA assay, titres of both ≥ 8 and ≥ 128 have been suggested as the correlate of protection [33]. Median rSBA titers were 2 for all serogroups tested, i.e. below the level of protection. rSBA titer ≥ 8 was detected in 6 (20.0%) individuals against MenA, 2 (6.7%) against MenC and 3 (10.0%) against MenW (Fig. 2). Among these, only 1 of 30 participants (3.3%) had rSBA titer ≥ 128 against MenA, MenC and MenW. For MenY, rSBA titer ≥ 128 was detected in 2 of the 12 individuals (16.7%) with clearly defined killing curves. Only two participants had protective rSBA titres ≥ 8 against two serogroups, one against MenW and MenY and the other against MenC and MenY. None of the participants had protective titres against more than two serogroups.

3.4. PS-specific antibodies in saliva

Anti-meningococcal PS IgA and IgG GMCs in saliva of the 1054 unvaccinated individuals per age, sex, and carriage of MenY are shown in Table 3. Anti-PS IgA GMCs showed a slightly increasing trend with age for all serogroups. The same pattern was not observed for anti-PS IgG GMCs, where there was a small dip in antibody levels in 16–18-year-olds. However, the absolute differences by age for both anti-PS IgA and IgG were small. As observed for anti-PS IgG GMCs in serum, anti-PS IgA and anti-PS IgG GMCs in saliva were similar between sexes. Anti-PS MenY IgA GMC was higher among the 19 carriers of MenY with saliva samples than among the non-carriers, while this difference was not observed for salivary anti-PS MenY IgG.

There were no major differences in anti-PS IgA and IgG GMCs between vaccinated and unvaccinated individuals among the 876 upper secondary school students (Table 2).

3.5. Correlations between PS-specific antibodies in serum and saliva

Among all unvaccinated individuals (carriers and non-carriers collectively), the correlation between meningococcal PS-specific antibody levels was low between serum and salivary IgG (Rho 0.1–0.2), serum IgG and salivary IgA (Rho 0.1–0.4), and between salivary IgA and IgG (Rho 0.3–0.4) for all serogroups. For the unvaccinated carriers of

Table 1
Geometric mean concentrations (GMCs) for anti-meningococcal serogroup A, C, W and Y polysaccharide-specific IgG in serum (n = 1109) by age, sex, and MenY carriage status among unvaccinated participants.

	n*	MenA GMC (95% CI)	n*	MenC GMC (95% CI)	n*	MenW GMC (95% CI)	n*	MenY GMC (95% CI)
Serum IgG (μg/mL)								
Age								
≤13	144	2.10 (1.79–2.47)	144	0.35 (0.26–0.49)	144	0.54 (0.45–0.65)	144	0.92 (0.75–1.13)
14	135	2.13 (1.67–2.72)	135	0.33 (0.23–0.47)	135	0.48 (0.37–0.63)	135	0.82 (0.60–1.12)
15	158	2.35 (1.95–2.83)	158	0.33 (0.27–0.41)	158	0.55 (0.46–0.65)	158	0.95 (0.78–1.15)
16	276	1.58 (1.44–1.74)	276	0.24 (0.20–0.29)	276	0.42 (0.34–0.52)	276	0.62 (0.55–0.70)
17	186	1.37 (1.21–1.57)	186	0.21 (0.17–0.26)	186	0.35 (0.31–0.39)	186	0.58 (0.48–0.70)
18	173	2.01 (1.69–2.39)	173	0.32 (0.25–0.42)	173	0.51 (0.37–0.70)	173	0.83 (0.66–1.04)
≥19	37	1.73 (1.23–2.44)	37	0.32 (0.23–0.44)	37	0.36 (0.20–0.64)	37	0.88 (0.36–2.17)
Sex								
Male	453	1.98 (1.73–2.28)	453	0.31 (0.26–0.37)	453	0.50 (0.42–0.58)	453	0.90 (0.76–1.06)
Female	656	1.73 (1.57–1.92)	656	0.27 (0.24–0.31)	656	0.43 (0.38–0.49)	656	0.66 (0.58–0.76)
Carriage								
MenY carrier	NA	NA	NA	NA	NA	NA	18	2.06 (0.89–4.79)
Not MenY carrier	NA	NA	NA	NA	NA	NA	1090	0.74 (0.66–0.83)

NA = not applicable.
* Number of samples with valid results per group analysed.

Table 2
Geometric mean concentrations (GMC) for anti-meningococcal serogroup A, C, W and Y polysaccharide specific IgG in serum (n = 890) and IgA and IgG in saliva (n = 876) by vaccination status among students in upper secondary schools.

	n*	MenA GMC (95% CI)	n*	MenC GMC (95% CI)	n*	MenW GMC (95% CI)	n*	MenY GMC (95% CI)
Serum IgG (µg/mL)								
Unvaccinated	686	1.63 (1.48–1.81)	686	0.26 (0.23–0.29)	686	0.42 (0.35–0.50)	686	0.67 (0.58–0.77)
Vaccinated	204	7.27 (5.90–8.95)	204	4.33 (3.38–5.55)	204	2.39 (1.52–3.76)	204	4.72 (3.31–6.73)
Saliva IgA (ng/mL)								
Unvaccinated	578	20.81 (17.96–24.12)	628	8.11 (7.02–9.37)	650	10.94 (9.60–12.47)	620	22.60 (18.94–26.96)
Vaccinated	188	23.18 (18.91–28.40)	198	10.45 (8.75–12.48)	204	14.78 (11.08–19.70)	204	28.01 (21.47–36.54)
Saliva IgG (ng/mL)								
Unvaccinated	543	101.22 (47.15–217.30)	516	3.30 (1.56–6.95)	490	3.98 (1.78–8.88)	570	7.40 (3.46–15.83)
Vaccinated	181	55.93 (23.15–135.11)	174	4.43 (2.68–7.32)	158	3.89 (2.26–6.68)	181	7.46 (4.23–13.16)

* Number of samples with valid results per group analysed.

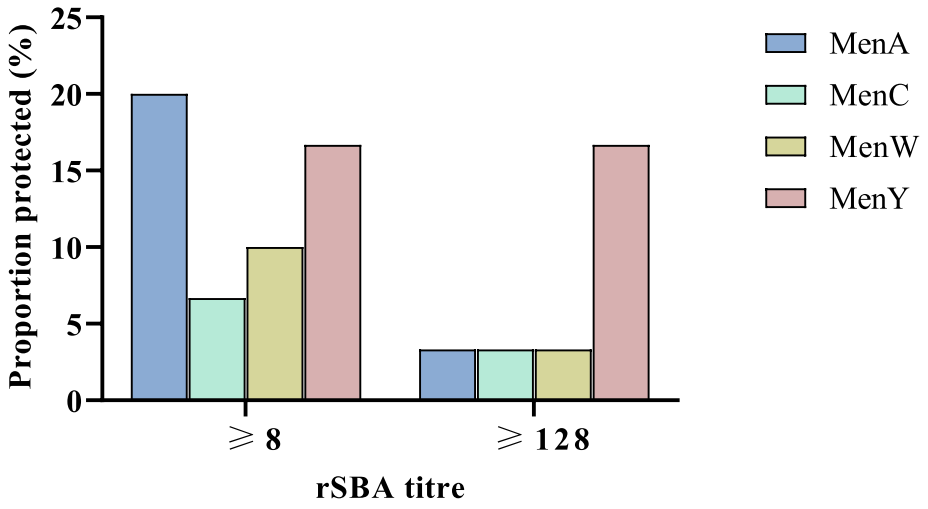

Fig. 2. Proportion of unvaccinated participants with baby rabbit serum bactericidal antibody (rSBA) levels ≥ 8 or ≥ 128 against meningococcal serogroup A (n = 30), C (n = 30), W (n = 30) and Y (n = 12).

MenY, anti-PS MenY IgG levels in serum were strongly correlated with anti-PS MenY IgA levels in saliva (Rho 0.8) (Fig. 3a), and moderately correlated with salivary anti-PS MenY IgG levels (Rho 0.6) (Fig. 3b). The correlation between anti-PS IgG in serum and rSBA in the unvaccinated non-carriers was low for all the four serogroups (Pearson's Rho 0.1–0.3).

3.6. Risk factors associated with differences in MenY antibody levels

We studied whether risk factors were associated with MenY PS-specific antibody levels in unvaccinated participants using multivariable analysis. Characteristics of the participants included in the analyses of risk factors are shown in Table 4.

Male sex was associated with higher anti-PS MenY serum IgG and salivary IgA levels compared to females (Table 5), but the absolute difference in concentration without correcting for possible confounders was small and the confidence intervals overlapping. A sex-dependent difference was not seen for anti-PS MenY salivary IgG. MenY carriers had higher levels of anti-PS MenY IgG in serum and IgA in saliva than non-carriers. The use of Swedish snus daily or occasionally was associated with lower anti-PS MenY IgG levels in serum. Daily or occasional use of waterpipe was associated with lower anti-PS MenY salivary IgG levels. Partying 7–10 times a week was associated with lower anti-PS MenY salivary IgA levels compared to not partying.

4. Discussion

Our results showed that anti-meningococcal PS antibody levels in serum and saliva were low among unvaccinated non-carriers for all serogroups and most of the subpopulation assessed with SBA did not have protective levels. MenY carriers had higher anti-PS MenY serum IgG and salivary IgA than those not carrying MenY. Use of Swedish snus was associated with lower serum anti-PS MenY IgG levels and use of waterpipe with lower salivary anti-PS MenY IgG levels. As expected, the serum anti-PS IgG levels were lower among the unvaccinated participants than the vaccinated for all serogroups, whereas the anti-PS IgA and IgG levels in saliva did not differ by vaccination status.

4.1. Antibody responses in serum

The anti-meningococcal PS MenA IgG levels in serum were in general higher than for the other tested serogroups, as reported by others [26]. This is probably a result of cross-protective antibodies through exposure to other bacteria with similar antigens as MenA [34–35]. Peltola and colleagues showed that 60% of the unvaccinated individuals had MenA serum anti-PS Ig levels considered as protective (≥2µg/mL measured by radioimmunoassay) [27]. Less than one in four in our study had equivalently high anti-PS IgG levels for MenC, MenW and MenY. In contrast, 56–90% of vaccinated individuals had serum anti-PS IgG above this level for all serogroups. This indicates that unvaccinated individuals have low degree of natural immunity against IMD caused by serogroups circulating in this

Table 3
Geometric mean concentrations (GMCs) for anti-meningococcal serogroup A, C, W and Y polysaccharide-specific IgA and IgG in saliva (n = 1054) by age, sex, and MenY carriage status among unvaccinated participants.

	n*	MenA GMC (95% CI)	n*	MenC GMC (95% CI)	n*	MenW GMC (95% CI)	n*	MenY GMC (95% CI)
Saliva IgA (ng/mL)								
Age								
≤13	108	11.46 (9.04–14.54)	108	6.00 (4.02–8.95)	112	7.28 (6.03–8.79)	112	14.43 (11.49–18.12)
14	108	13.88 (11.84–16.28)	109	6.90 (5.33–8.92)	111	8.16 (6.73–9.89)	107	17.49 (15.19–20.15)
15	124	13.72 (11.93–15.77)	117	5.78 (4.38–7.63)	125	8.45 (7.19–9.94)	122	17.77 (14.76–21.40)
16	225	20.41 (17.55–23.73)	248	8.08 (6.73–9.70)	261	11.10 (9.59–12.85)	245	22.38 (18.16–27.58)
17	161	18.45 (16.10–21.16)	171	6.55 (5.29–8.11)	177	9.25 (7.70–11.12)	173	19.41 (16.18–23.28)
18	149	22.18 (16.89–29.12)	163	9.30 (7.65–11.31)	168	11.64 (10.35–13.10)	157	23.77 (17.96–31.46)
≥19	32	32.03 (25.08–40.91)	33	13.27 (9.35–18.83)	30	14.98 (11.65–19.27)	33	36.56 (25.20–53.05)
Sex								
Male	376	17.36 (14.08–21.40)	378	8.13 (6.64–9.94)	389	10.20 (8.64–12.05)	379	21.98 (18.12–26.67)
Female	531	17.49 (15.31–19.99)	571	6.93 (6.12–7.86)	595	9.42 (8.46–10.48)	570	18.92 (16.63–21.53)
Carriage								
MenY carrier	NA	NA	NA	NA	NA	NA	19	50.16 (29.98–83.92)
Not MenY carrier	NA	NA	NA	NA	NA	NA	929	19.70 (17.09–22.71)
Saliva IgG (ng/mL)								
Age								
≤13	113	157.24 (82.85–298.41)	117	6.37 (3.04–13.37)	108	9.81 (5.09–18.90)	107	21.41 (12.74–35.98)
14	102	174.50 (90.63–335.97)	109	8.09 (4.25–15.43)	105	13.66 (8.16–22.88)	100	28.59 (18.03–45.33)
15	115	164.23 (93.18–289.45)	120	7.05 (3.45–14.39)	105	12.26 (6.63–22.66)	108	24.77 (14.29–42.94)
16	225	116.80 (52.35–260.63)	204	3.11 (1.24–7.81)	180	3.69 (1.47–9.26)	230	7.71 (3.43–17.31)
17	146	70.01 (35.33–138.74)	149	2.52 (1.46–4.36)	141	3.15 (1.65–5.99)	158	5.43 (2.92–10.11)
18	132	88.71 (35.01–224.81)	124	3.79 (1.64–8.78)	132	4.19 (1.82–9.61)	140	7.18 (3.32–15.51)
≥19	29	368.41 (105.19–1290.37)	29	9.29 (2.24–38.45)	29	12.07 (1.51–96.54)	32	26.64 (4.58–155.04)
Sex								
Male	350	113.68 (64.52–200.29)	349	4.77 (2.77–8.22)	329	6.84 (3.78–12.38)	361	13.87 (7.81–24.62)
Female	512	127.66 (76.58–212.78)	503	4.30 (2.55–7.25)	471	5.58 (3.21–9.70)	514	9.90 (5.76–17.04)
Carriage								
MenY carrier	NA	NA	NA	NA	NA	NA	11	11.37 (6.67–19.37)
Not MenY carrier	NA	NA	NA	NA	NA	NA	863	8.34 (1.11–62.79)

NA = not applicable.
* Number of samples with valid results per group analysed.

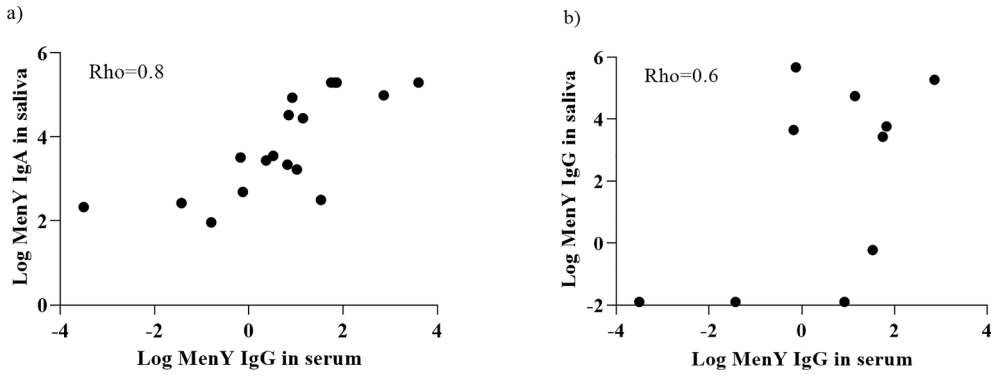

Fig. 3. Correlation between anti-meningococcal serogroup Y polysaccharide-specific IgG in serum and a) IgA in saliva (n = 18) and b) IgG in saliva (n = 10) among unvaccinated carriers of serogroup Y *N. meningitidis* using Pearson's correlation coefficient (Rho).

age group in Norway and that natural immunity is not inducing adequate protection against IMD. However, the level of ≥ 2 μg/mL was only suggested protective for MenA [27]. A Dutch study has shown that adolescents with anti-PS IgG levels below 2 μg/mL for serogroups A, W and Y had high SBA titres against the same serogroups [36], indicating that the protective level for anti-PS IgG in serum might be lower than 2 μg/mL.

The multivariable analysis showed that users of Swedish snus had lower anti-meningococcal PS MenY IgG levels in serum compared to non-users. Nicotine has been shown to reduce both humoral and cellular immunity [37], which supports our findings. We did not find a similar association for cigarette smokers or e-cigarette users. However, only 3.8% of the cigarette smokers and 9.9% of the e-cigarette users were daily users as opposed to 51.7% of the Swedish snus users. Moreover, Swedish snus users could be exposed to higher plasma concentrations of nicotine [37–41] which might contribute to the difference in antibody levels.

Table 4
Characteristics of the unvaccinated participants assessed for association of risk factors and meningococcal serogroup Y polysaccharide-specific antibodies in serum and saliva. Based on complete questionnaire data from 1068 participants with serum samples and 1018 participants with saliva samples.

Variable	Response	Participants with serum samples (%)	Participants with saliva samples (%)
Sex	Male	432 (40.5)	410 (40.3)
	Female	636 (59.6)	608 (59.7)
Age	≤13	141 (13.2)	132 (13.0)
	14	131 (12.3)	120 (11.8)
	15	150 (14.0)	134 (13.2)
	16	260 (24.3)	253 (24.9)
	17	183 (17.1)	178 (17.5)
	18	170 (15.9)	169 (16.6)
	≥19	33 (3.1)	32 (3.1)
Carriage of genogroup Y *N. meningitidis*	No	1051 (98.4)	1000 (98.2)
	Yes	17 (1.6)	18 (1.8)
Use of Swedish snus[†]	No	921 (86.2)	878 (86.3)
	Yes	147 (13.8)	140 (13.8)
Cigarette smoker	No	929 (87.0)	887 (87.1)
	Yes	139 (13.0)	131 (12.9)
Exposure to passive smoking	No	908 (85.0)	869 (85.4)
	Yes	160 (15.0)	149 (14.6)
E-cigarette smoker	No	1030 (96.4)	980 (96.3)
	Yes	38 (3.6)	38 (3.7)
Use of waterpipe	No	1042 (97.6)	992 (97.5)
	Yes	26 (2.4)	26 (2.6)
Times attended social events[‡] last 3 months	None	315 (29.5)	287 (28.2)
	1–3 times	418 (39.1)	405 (39.8)
	4–6 times	173 (16.2)	166 (16.3)
	7–10 times	92 (8.6)	91 (8.9)
	> 10 times	70 (6.6)	69 (6.8)
Throat pain/upper respiratory infection previous week	No	678 (63.5)	647 (63.6)
	Yes	390 (36.5)	371 (36.4)

[†] Smokeless tobacco.
[‡] Attending parties, bars, or big youth arrangements.

4.2. Antibody responses in saliva

Among the unvaccinated participants, there was a slight increase of salivary anti-meningococcal PS IgA with age for all serogroups, as seen in a study among children, college students and adults in the UK [42]. For MenY, this could be a result of increasing carriage of genogroup Y with age previously found among the study participants [9]. As observed in serum of the unvaccinated individuals, salivary anti-PS IgA levels against MenY were higher among carriers of MenY than among those not carrying MenY. A similar relationship between anti-MenB salivary IgA and MenB carriage was reported in the British study [42]. The same carriage-associated pattern was not seen for anti-PS MenY IgG in saliva. IgA is the dominating immunoglobulin on mucosal surfaces [43], whereas salivary IgG predominantly originates from transudate of serum over the mucosal barrier and is not produced locally [44]. Our results support that carriage-induced local immunity in unvaccinated individuals is driven by mucosal IgA production.

Both anti-PS IgA and IgG in saliva are induced after vaccination with meningococcal conjugate vaccines [45], which could imply that in vaccinated individuals, IgG also contributes to prevention of meningococcal attachment and colonisation. We did not detect any differences in PS-specific antibodies in saliva between unvaccinated and vaccinated individuals. The median time since vaccination in the vaccinated individuals in our study was almost one year. A Dutch study among adolescents vaccinated with MCV4 found that salivary anti- PS IgA and IgG levels were increased one month after vaccination but were comparable to pre-vaccination levels after one year [25]. This suggests that vaccine-induced local immunity is short-lived.

Use of Swedish snus was associated with lower anti-meningococcal PS IgG levels in serum. However, we did not find a similar association for salivary anti-PS IgA. We have previously shown that the use of Swedish snus is associated with higher meningococcal carriage rate [9], and in this study, we found that carriage was associated with higher anti-PS IgA in saliva. Thus, the negative effect of nicotine on antibody production may be counteracted by the antibody-inducing effect of carriage. A British study found a relationship between smoking and higher anti-PS IgA in saliva independent of carriage [42]. Nonetheless, we did not detect an equivalent association with smoking perhaps due to the low number of daily smokers.

Table 5
Multivariable analysis of the association between anti-meningococcal serogroup Y polysaccharide-specific IgG levels in serum and IgA and IgG levels in saliva, and risk factors among unvaccinated individuals. Based on complete questionnaire data from 1068 participants with serum samples and 1018 participants with saliva samples.

		Serum IgG	Saliva IgA	Saliva IgG
Variable		Coefficient (95% CI)	Coefficient (95% CI)	Coefficient (95% CI)
Sex	Male	1 (Ref)	1 (Ref)	1 (Ref)
	Female	−0.27 (−0.45–−0.10)	−0.15 (−0.28–−0.02)	−0.08 (−0.41–0.24)
Carriage of genogroup Y *N. meningitidis*	No	1 (Ref)	1 (Ref)	1 (Ref)
	Yes	1.23 (0.34–2.11)	1.05 (0.56–1.54)	− 0.47 (−2.17–1.22)
Use of Swedish snus[†]	No	1 (Ref)	1 (Ref)	1 (Ref)
	Yes	−0.31 (−0.53–−0.10)	0.08 (−0.10–0.27)	−0.13 (−0.56–0.31)
Cigarette smoker	No	1 (Ref)	1 (Ref)	1 (Ref)
	Yes	0.08 (−0.13–0.29)	0.05 (−0.12–0.223	0.37 (−0.12–0.86)
Exposure to passive smoking	No	1 (Ref)	1 (Ref)	1 (Ref)
	Yes	0.10 (−0.18–0.39)	0.01 (−0.18–0.21)	0.25 (−0.06–0.56)
E-cigarette smoker	No	1 (Ref)	1 (Ref)	1 (Ref)
	Yes	0.05 (−0.42–0.52)	−0.05 (−0.37–0.28)	−0.12 (−0.96–0.73)
Use of waterpipe	No	1 (Ref)	1 (Ref)	1 (Ref)
	Yes	−0.52 (−1.23–0.19)	−0.29 (−0.62–0.03)	−1.24 (−2.10–−0.37)
Times attended social events[‡] last 3 months	None	1 (Ref)	1 (Ref)	1 (Ref)
	1–3 times	−0.05 (−0.24–0.15)	−0.05 (−0.15–0.05)	0.21 (−0.10–0.51)
	4–6 times	−0.08 (−0.34–0.17)	−0.13 (−0.34–0.07)	−0.32 (−0.82–0.18)
	7–10 times	−0.18 (−0.57–0.22)	−0.28 (−0.54–−0.01)	−0.42 (−1.05–0.21)
	> 10 times	−0.08 (−0.45–0.30)	−0.23 (−0.60–0.14)	−0.16 (−0.71–0.39)
Throat pain/upper respiratory infection previous week	No	1 (Ref)	1 (Ref)	1 (Ref)
	Yes	−0.02 (−0.22–0.18)	−0.01 (−0.10–0.08)	0.21 (−0.14–0.56)

[†] Smokeless tobacco.
[‡] Attending parties, bars, or big youth arrangements.

4.3. Strengths and limitations

The strengths of our study were the large number of participants and the investigation of both mucosal and systemic immunity against meningococci. A limitation to our study was that a correlate of protection against IMD based on specific serum Ig has only been suggested for MenA [27], and the level of protection against other serogroups might be different. However, as the anti-PS IgG levels in serum among the unvaccinated were low and the subset tested for SBA confirmed low degree of protection, even in those within the higher PS IgG range, it is likely that most of the unvaccinated individuals in our study were not adequately protected against IMD caused by serogroups C, W and Y. Since no carriers were included in the SBA analysis, we could not assess if the higher anti-PS MenY IgG levels among carriers of MenY were protective. A protective threshold has been suggested for MenC, MenW and MenY salivary IgG [46]. However, the thresholds were found to be less accurate in unvaccinated individuals which was the focus in this study.

5. Conclusion

Unvaccinated adolescents in Norway have low levels of meningococcal PS-specific antibodies in serum and saliva against the predominant serogroups causing IMD in this age group. Carriage of *N. meningitidis* serogroup Y increases levels of IgG in serum and IgA in saliva against the homologous serogroup. However, natural immunity has only minor impact on antibody levels in serum among adolescents compared with vaccination with MCV4. Introduction of MCV4 in the national immunisation program is therefore recommended.

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Authors contributions

All authors attest they meet the ICMJE criteria for authorship. SVW, LMN, DAC and GT initiated and designed the study. GT, LMN and SVW participated in the sampling. IL, SVW and GT performed the statistical analyses. TB was head of data management. BB, MBH and DBB performed the serum- and saliva analyses. SVW drafted the manuscript. All authors contributed to the interpretation of the data, to writing and revising the manuscript and to approval of the final manuscript.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Sara Viksmoen Watle reports financial support was provided by Renée and Bredo Grimsgaard Foundation.

Data availability

The authors do not have permission to share data.

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References

[1] Wang B, Santoreneos R, Giles L, Haji Ali Afzali H, Marshall H. Case fatality rates of invasive meningococcal disease by serogroup and age: A systematic review and meta-analysis. Vaccine 2019;37(21):2768–82. https://doi.org/10.1016/j.vaccine.2019.04.020.
[2] Olbrich KJ, Muller D, Schumacher S, Beck E, Meszaros K, Koerber F. Systematic review of invasive meningococcal disease: Sequelae and quality of life impact on patients and their caregivers. Infect Dis Ther 2018;7(4):421–38. https://doi.org/10.1007/s40121-018-0213-2.
[3] Agarwal S, Vasudhev S, DeOliveira RB, Ram S. Inhibition of the classical pathway of complement by meningococcal capsular polysaccharides. J Immunol 2014;193(4):1855–63. https://doi.org/10.4049/jimmunol.1303177.
[4] Christensen H, May M, Bowen L, Hickman M, Trotter CL. Meningococcal carriage by age: A systematic review and meta-analysis. Lancet Infect Dis 2010;10(12):853–61. https://doi.org/10.1016/s1473-3099(10)70251-6.
[5] Peterson ME, Mile R, Li Y, Nair H, Kyaw MH. Meningococcal carriage in high-risk settings: A systematic review. Int J Infect Dis 2018;73:109–17. https://doi.org/10.1016/j.ijid.2018.05.022.
[6] MacLennan JM, Rodrigues CMC, Bratcher HB, Lekshmi A, Finn A, Oliver J, et al. Meningococcal carriage in periods of high and low invasive meningococcal disease incidence in the UK: Comparison of UKMenCar1-4 cross-sectional survey results. Lancet Infect Dis 2021;21(5):677–87. https://doi.org/10.1016/s1473-3099(20)30842-2.
[7] Säll O, Lorraine E, Berhane AI, Alexander P, Anders M, Sara TH, et al. Prevalence and persistence of *Neisseria meningitidis* carriage in Swedish university students. Epidemiol Infect 2023;151:e25.
[8] Norwegian Institute of Public Health. Norwegian surveillance system for communicable diseases. Oslo, Norway [cited 22 June 2023]. Available from: http://msis.no/.
[9] Watle SV, Caugant DA, Tunheim G, Bekkevold T, Laake I, Brynildsrud OB, et al. Meningococcal carriage in Norwegian teenagers: Strain characterisation and assessment of risk factors. Epidemiol Infect 2020;148:e80.
[10] Fjaer EG, Pedersen W, Sandberg S. Party on wheels: Mobile party spaces in the Norwegian high school graduation celebration. Br J Sociol 2016;67(2):328–47. https://doi.org/10.1111/1468-4446.12189.
[11] Norwegian Institute of Public Health. Norwegian immunisation registry SYSVAK. Oslo, Norway [cited 23 June 2023]. Available from: https://www.fhi.no/en/hn/health-registries/norwegian-immunisation-registry-sysvak/.
[12] Mueller JE, Yaro S, Njanpop-Lafourcade BM, Drabo A, Idohou RS, Kroman SS, et al. Study of a localized meningococcal meningitis epidemic in Burkina Faso: Incidence, carriage, and immunity. J Infect Dis 2011;204(11):1787–95. https://doi.org/10.1093/infdis/jir623.
[13] Dale AP, Theodosiou AA, Gbesemete DF, Guy JM, Jones EF, Hill AR, et al. Effect of colonisation with *Neisseria lactamica* on cross-reactive anti-meningococcal B-cell responses: A randomised, controlled, human infection trial. Lancet Microbe 2022;3(12):e931–43. https://doi.org/10.1016/s2666-5247(22)00283-x.
[14] Evans CM, Pratt CB, Matheson M, Vaughan TE, Findlow J, Borrow R, et al. Nasopharyngeal colonization by *Neisseria lactamica* and induction of protective immunity against *Neisseria meningitidis*. Clin Infect Dis 2011;52(1):70–7. https://doi.org/10.1093/cid/ciq065.
[15] Reller LB, MacGregor RR, Beaty HN. Bactericidal antibody after colonization with *Neisseria meningitidis*. J Infect Dis 1973;127(1):56–62. https://doi.org/10.1093/infdis/127.1.56.
[16] Pollard AJ, Frasch C. Development of natural immunity to *Neisseria meningitidis*. Vaccine 2001;19(11):1327–46. https://doi.org/10.1016/S0264-410X(00)00333-9.
[17] Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus. II. Development of natural immunity. J Exp Med 1969;129(6):1327–48. https://doi.org/10.1084/jem.129.6.1327.
[18] Pichichero ME. Booster vaccinations: Can immunologic memory outpace disease pathogenesis? Pediatrics 2009;124(6):1633–41. https://doi.org/10.1542/peds.2008-3645.
[19] Clark SA, Borrow R. Herd protection against meningococcal disease through vaccination. Microorganisms 2020;8(11). https://doi.org/10.3390/microorganisms8111675.
[20] Erlich KS, Congeni BL. Importance of circulating antibodies in protection against meningococcal disease. Hum Vaccin Immunother 2012;8(8):1029–35. https://doi.org/10.4161/hv.20473.
[21] Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus. I. The role of humoral antibodies. J Exp Med 1969;129(6):1307–26. https://doi.org/10.1084/jem.129.6.1307.
[22] European Medicines Agency. EMEA/H/C/001095: Assessment report for Menveo. [cited 18 August 2023]. Available from: https://www.ema.europa.eu/en/documents/assessment-report/menveo-epar-public-assessment-report_en.pdf.
[23] European Medicines Agency. EMEA/H/C/002226: Assessment report for Nimenrix. [cited 18 August 2023]. Available from: https://www.ema.europa.eu/en/documents/assessment-report/nimenrix-epar-public-assessment-report_en.pdf.
[24] Bårnes GK, Workalemahu B, Kristiansen PA, Beyene D, Merdekios B, Fissiha P, et al. Salivary and serum antibody response against *Neisseria meningitidis* after

vaccination with conjugate polysaccharide vaccines in Ethiopian volunteers. Scand J Immunol 2016;84(2):118–29. https://doi.org/10.1111/sji.12451.
[25] van Ravenhorst MB, den Hartog G, van der Klis FRM, van Rooijen DM, Sanders EAM, Berbers GAM. Induction of salivary antibody levels in Dutch adolescents after immunization with monovalent meningococcal serogroup C or quadrivalent meningococcal serogroup A, C, W and Y conjugate vaccine. PLoS One 2018;13(4):e0191261.
[26] Ohm M, Knol MJ, Vos ERA, Bogaard MJM, van Rooijen DM, Sanders EAM, et al. Seroprevalence of meningococcal ACWY antibodies across the population in the Netherlands: Two consecutive surveys in 2016/17 and 2020. Vaccine 2022;40(1):59–66. https://doi.org/10.1016/j.vaccine.2021.11.045.
[27] Peltola H, Mäkelä H, Käyhty H, Jousimies H, Herva E, Hällström K, et al. Clinical efficacy of meningococcus group A capsular polysaccharide vaccine in children three months to five years of age. N Engl J Med 1977;297(13):686–91. https://doi.org/10.1056/nejm197709292971302.
[28] Barnes GK, Kristiansen PA, Caugant DA, Naess LM. Development and evaluation of a multiplex microsphere assay for quantitation of IgG and IgA antibodies against *Neisseria meningitidis* serogroup A, C, W and Y polysaccharides. Clin Vaccine Immunol 2015;22(7):697–705. https://doi.org/10.1128/cvi.00087-15.
[29] Stoof SP, van der Klis FRM, van Rooijen DM, Bogaert D, Trzciński K, Sanders EAM, et al. Salivary antibody levels in adolescents in response to a meningococcal serogroup C conjugate booster vaccination nine years after priming: Systemically induced local immunity and saliva as potential surveillance tool. Vaccine 2015;33(32):3933–9. https://doi.org/10.1016/j.vaccine.2015.06.055.
[30] Lucidarme J, Louth J, Townsend-Payne K, Borrow R. Meningococcal serogroup A, B, C, W, X, and Y serum bactericidal antibody assays. Methods Mol Biol 2019;1969:169–79. https://doi.org/10.1007/978-1-4939-9202-7_12.
[31] Maslanka SE, Gheesling LL, Libutti DE, Donaldson KB, Harakeh HS, Dykes JK, et al. Standardization and a multilaboratory comparison of *Neisseria meningitidis* serogroup A and C serum bactericidal assays. The multilaboratory study group. Clin Diagn Lab Immunol 1997;4(2):156–67. https://doi.org/10.1128/cdli.4.2.156-167.1997.
[32] Hanley JA, Negassa A, Edwardes MD, Forrester JE. Statistical analysis of correlated data using generalized estimating equations: An orientation. Am J Epidemiol 2003;157(4):364–75. https://doi.org/10.1093/aje/kwf215.
[33] McIntosh ED, Bröker M, Wassil J, Welsch JA, Borrow R. Serum bactericidal antibody assays - the role of complement in infection and immunity. Vaccine 2015;33(36):4414–21. https://doi.org/10.1016/j.vaccine.2015.07.019.
[34] Vann WF, Liu TY, Robbins JB. *Bacillus pumilus* polysaccharide cross-reactive with meningococcal group A polysaccharide. Infect Immun 1976;13(6):1654–62. https://doi.org/10.1128/iai.13.6.1654-1662.1976.
[35] Grados O, Ewing WH. Antigenic relationship between *Escherichia coli* and *Neisseria meningitidis*. J Infect Dis 1970;122(1):100–3. https://doi.org/10.1093/infdis/122.1-2.100.
[36] van Ravenhorst MB, van der Klis FRM, van Rooijen DM, Sanders EAM, Berbers GAM. Adolescent meningococcal serogroup A, W and Y immune responses following immunization with quadrivalent meningococcal A, C, W and Y conjugate vaccine: Optimal age for vaccination. Vaccine 2017;35(36):4753–60. https://doi.org/10.1016/j.vaccine.2017.06.007.
[37] Sopori ML, Razani-Boroujerdi S, Singh SP. Immunomodulatory effects of cigarette smoke/nicotine. In: Friedman H, Klein TW, Bendinelli M, editors. Infectious diseases and substance abuse. Infectious agents and pathogenesis. Boston, MA: Springer US; 2005. p. 103–9. Available from: https://doi.org/10.1007/0-306-48688-1_8.
[38] Bekki K, Inaba Y, Uchiyama S, Kunugita N. Comparison of chemicals in mainstream smoke in heat-not-burn tobacco and combustion cigarettes. J uoeh 2017;39(3):201–7. https://doi.org/10.7888/juoeh.39.201.
[39] Farsalinos KE, Yannovits N, Sarri T, Voudris V, Poulas K. Nicotine delivery to the aerosol of a heat-not-burn tobacco product: Comparison with a tobacco cigarette and e-cigarettes. Nicotine Tob Res 2018;20(8):1004–9. https://doi.org/10.1093/ntr/ntx138.
[40] Digard H, Proctor C, Kulasekaran A, Malmqvist U, Richter A. Determination of nicotine absorption from multiple tobacco products and nicotine gum. Nicotine Tob Res 2013;15(1):255–61. https://doi.org/10.1093/ntr/nts123.
[41] Vedøy TF, Lund KE. Nicotine content in Swedish-type snus sold in Norway from 2005 to 2020. Nicotine Tob Res 2022;24(7):1130–3. https://doi.org/10.1093/ntr/ntac006.
[42] Horton RE, Stuart J, Christensen H, Borrow R, Guthrie T, Davenport V, et al. Influence of age and carriage status on salivary IgA to *Neisseria meningitidis*. Epidemiol Infect 2005;133(5):883–9. https://doi.org/10.1017/S0950268805004097.
[43] Li Y, Jin L, Chen T. The effects of secretory IgA in the mucosal immune system. Biomed Res Int 2020;2020:2032057. https://doi.org/10.1155/2020/2032057.
[44] Brandtzaeg P. Secretory immunity with special reference to the oral cavity. J Oral Microbiol 2013;5. https://doi.org/10.3402/jom.v5i0.20401.
[45] Zhang Q, Finn A. Mucosal immunology of vaccines against pathogenic nasopharyngeal bacteria. J Clin Pathol 2004;57(10):1015–21. https://doi.org/10.1136/jcp.2004.016253.
[46] van Ravenhorst MB, van der Klis FRM, van Rooijen DM, Sanders EAM, Berbers GAM. Use of saliva to monitor meningococcal vaccine responses: Proposing a threshold in saliva as surrogate of protection. BMC Med Res Methodol 2019;19(1):1. https://doi.org/10.1186/s12874-018-0650-3.