VACCINE-INDUCED IMMUNITY AND PROTECTION AGAINST INVASIVE MENINGOCOCCAL SEROGROUP ACWY DISEASE IN THE NETHERLANDS

MILOU OHM

Vaccine-induced immunity and protection against invasive meningococcal serogroup ACWY disease in the Netherlands

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Vaccin-geïnduceerde immuniteit en bescherming tegen invasieve meningokokken serogroep ACWY ziekte in Nederland
(met een samenvatting in het Nederlands)

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Promotor:
Prof. dr. E.A.M. Sanders

Copromotoren:
Dr. W.A.M. Berbers
Dr. M.J. Knol

Beoordelingscommissie:
Prof. dr. D. van de Beek
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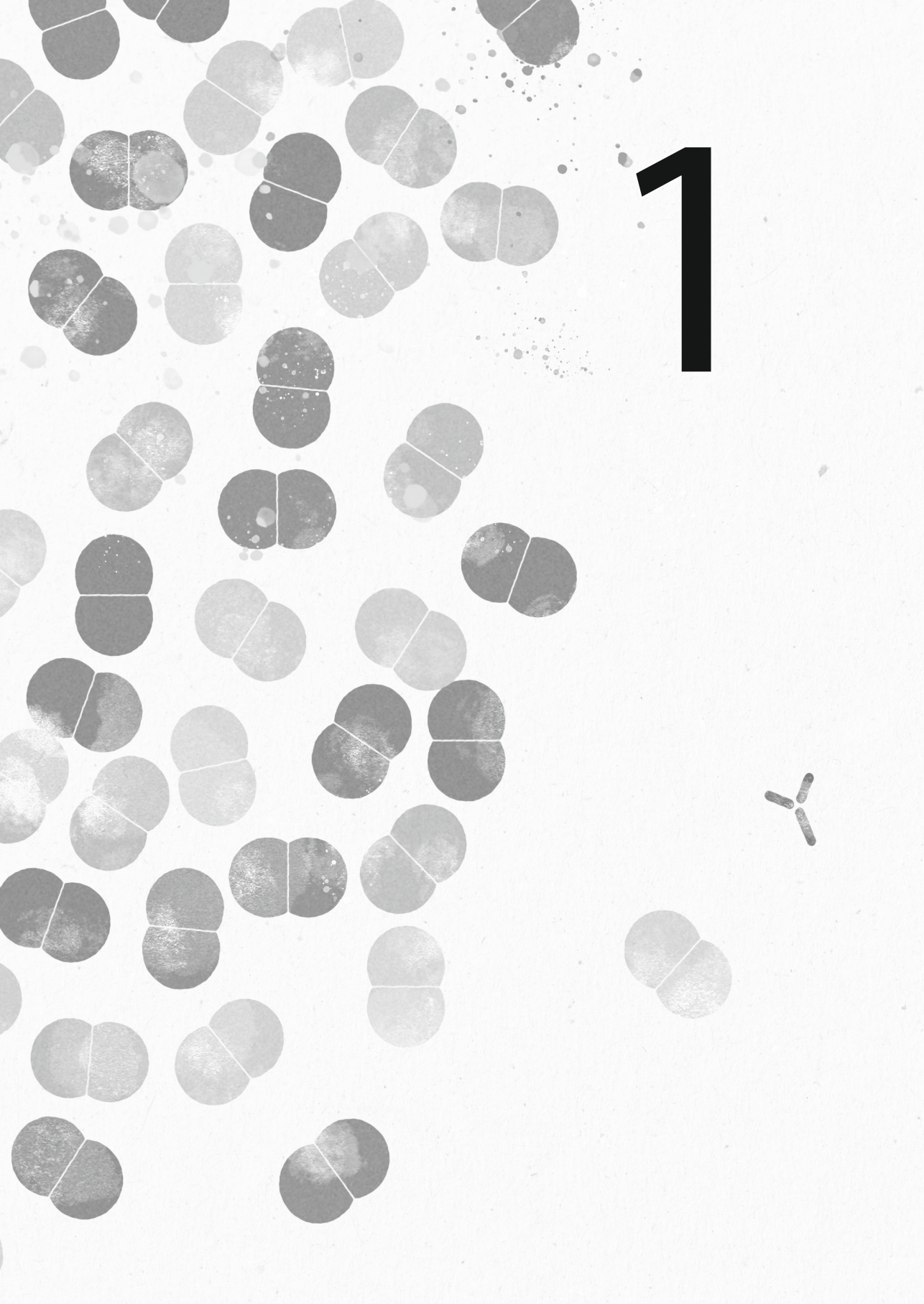
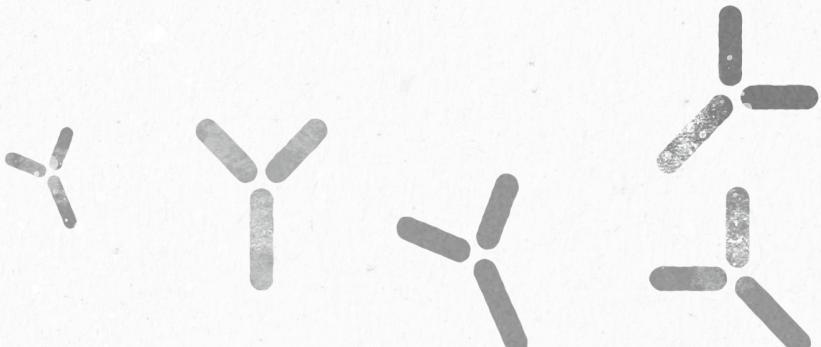


General introduction



The meningococcal bacterium, *Neisseria meningitidis*, is a Gram-negative diplococcus that exclusively infects humans [1]. It was first identified and cultured in cerebrospinal fluid from a patient with meningitis by Weichselbaum in 1887 [2]. Hence, the etiologic relationship between the micro-organism – at that time called *Diplococcus intracellularis meningitidis* - and epidemic meningitis was established. *N. meningitidis* is either encapsulated or unencapsulated, but without capsule it rarely causes invasive disease. At present, 13 serogroups have been distinguished based on the polysaccharide capsule [3]. Healthy individuals may carry the bacterium in the upper respiratory tract (naso- and oropharynx) without experiencing symptoms. Colonization may instigate an immune response both locally and systemically including the production of antibodies directed to the polysaccharide capsule as well as to other antigens on the outer surface of the bacterium [4, 5]. While meningococcal carriage is quite common, the meningococcal bacterium rarely crosses the mucosal barriers. The prevalence of meningococcal carriage varies with age; rates are low in infants and adults (4-8%) and increase during childhood with highest carriage rates (up to 24%) estimated among adolescents [6]. Transmission may occur among close contacts through respiratory droplets and in addition to crowding, smoking and kissing have been described as risk factors [7, 8]. However, it is important to take into account that differences in age distribution and risk factors between continents exist. In the African so-called meningitis belt, meningococcal carriage was common among young children [9]. Furthermore, a seasonal effect on disease rates (epidemics during the dry season) was observed, although the limited data available about the association between season and carriage did not reach significance [9].

Invasive meningococcal disease

The unlikely event of meningococci passing the epithelial barrier usually occurs within 10 days from new-onset colonization [10]. The rapid progression of disease can lead to a life-threatening situation within hours after onset of symptoms. The predominant clinical manifestations of invasive meningococcal disease (IMD) include meningitis or sepsis, and both conditions may be featured simultaneously [11]. Other manifestations such as arthritis, pneumonia, or gastro-enteritis and chronic meningococcemia are less common and might not always be recognized immediately as meningococcal disease. Immediate antibiotic treatment is crucial to impede a deterioration of disease course, but lack of early diagnosis might impair immediate adequate medical support. Mortality rates up to ~25% have been reported in teenagers, particularly for septic shock, although case fatality rates are usually reported to be around 10% [12]. Fatality is associated with variables such as genetic factors and medical condition of the host, but many host (and also bacterial) factors are incompletely understood. Severe sequelae such as limb amputation, deafness and neurological deficits, but also a wide range of psychological and behavioral problems occur in a substantial part of survivors [13].

Global disease burden

Incidence rates of IMD vary widely across the world (Figure 1) and the geographical distribution of different serogroups changes over time. In the last century, hyperendemic situations and large epidemics were caused not by one specific, but rather by several different serogroups. In Africa, several serogroup A epidemics – mainly in the meningitis belt –have occurred with reported incidence rates up to 1,000 per 100,000 inhabitants [14]. Between 2010-16, serogroup B predominated in a large part of the world including Europe, Australia and New-Zealand, the United States and a substantial part of Latin America [15]. Historically, serogroup C was an important cause of disease in Europe but large vaccination campaigns targeting this serogroup decreased the prevalence of serogroup C to being virtually absent [16, 17]. From 2014 onwards, serogroup W has been the primary concern in Europe with dominance of a specific clonal complex (cc); the hypervirulent cc11 (also see: the meningococcal bacterium) [18, 19]. Prior to this increase in Europe, serogroup W meningococci belonging to cc11 caused an epidemic among Hajj pilgrims in Saudi Arabia - the strain known as the Hajj clone - in 2000, whereafter serogroup W became endemic or caused outbreaks for example in South America, United States, and China [20].

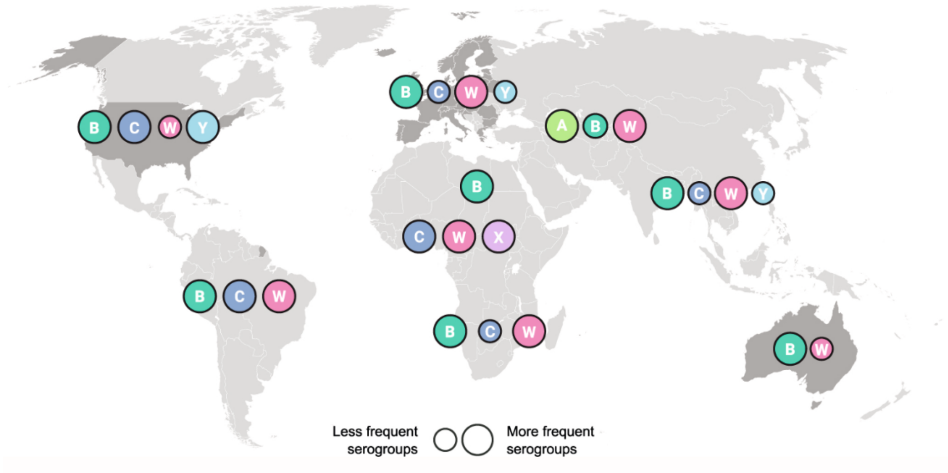


Figure 1. Worldwide serogroup distribution of invasive meningococcal disease in 2019 [21].

The meningococcal bacterium

Meningococci can be classified by the serogroup (capsule), the sero(sub)type, the immunotype (Figure 2) and in addition to that may also genetically belong to a specific sequence type. The capsular genes are located in the *cps* locus which is a single chromosomal locus divided into six regions. These regions encode different processes necessary for capsule formation, for instance biosynthesis of the capsule polysaccharide and translocation of the polysaccharide to surface of the cell [3]. The serotype is determined by one of the outer membrane proteins, the porin PorB, while another porin (PorA) determines the serosubtype. The immunotype can be assigned based on the structure of the lipooligosaccharide (LOS).

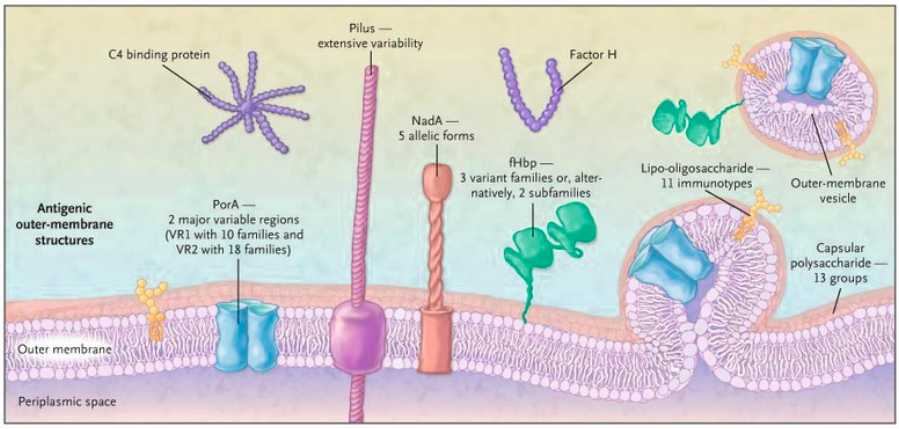


Figure 2. The structure of the meningococcal outer membrane, including the capsular polysaccharide and antigenic outer-membrane structures such as porin A (por A), Neisseria adhesin A (NadA), factor H binding protein (fHbp), lipo-oligosaccharide (LOS) [22].

To distinguish between different meningococcal strains/characterize isolates, seven housekeeping genes [24] that are required for maintaining basic cellular functions are used to assign an allelic profile based on the sequence type: the clonal complex. When a strain is of a specific cc, the housekeeping genes may be different, but should be closely related with a recognizable common origin [25]. Based on this approach called multilocus sequence typing (MLST), the use of typing schemes (pubmlst.org) enabled tracking of specific outbreak-causing isolates. The few, specific lineages that cause most outbreaks of invasive disease are called hyperinvasive lineages. Genomic surveillance – currently with whole genome sequencing (WGS) available as the most effective method – can establish relatedness between outbreak-causing strains and for example revealed that the MenW cc11 outbreak strain spreading throughout Europe (and South America) was distant from the strain responsible for the Hajj outbreak [26]. Similarities that were found between the outbreak in England and the Netherlands suggested that the evolution of IMD-W epidemiology in the Netherlands could be predicted by extrapolating from the disease trends in England [18]. This highlights the importance of genetics to describe population biology and to understand epidemiology.

Antibodies, the complement system and protection

The bacterial outer surface antigens as described above (see: the meningococcal bacterium), such as the polysaccharide capsule, are recognized by the immune system [27]. After recognition, a primary, innate response will be established including the

complement system that plays a major role in the protection against meningococci [28]. As soon as the complement system is activated, these proteins can improve the defense mechanism by opsonization, thereby facilitating phagocytosis. Moreover, complement can form a pore in the membrane by the membrane-attack complex (C5 – C9) and kill the bacterium by disrupting the thick protective bacterial polysaccharide wall that usually protects against various immune mechanisms [28]. Individuals that have a deficiency in the complement system are predisposed to (recurrent) IMD [28]. A more targeted response will be in place when the adaptive response occurs [29]. Lymphocytes with B- and T-cell receptors that recognize the micro-organism will proliferate into effector lymphocytes. These cells are involved in clearing the infection, as well as countering a potential subsequent infection by immunological memory. Activated B-cells produce antibodies after antigen recognition. Since antibodies may opsonize the bacterium and also induce complement-mediated killing, the optimal protection against IMD is provided when innate and adaptive systems interact (Figure 3).

There are several classes of antibody (isotypes), of which immunoglobulin G (IgG) is the most abundant in blood. IgG is a monomer that has two antigen binding sites and consists of four types (IgG1-IgG4). IgG is tailor-made after encountering an antigen and facilitates phagocytosis. Furthermore, IgG is important in the long term response, while the pentameric IgM provides an immediate immune response and has great potential to fix complement [31]. The dimer IgA is found in secretions such as saliva and is mainly present in mucosal areas but also to a lesser extent in serum. At the mucosal surface, it can provide a local response that facilitates the elimination of bugs thereby preventing invasion [32].

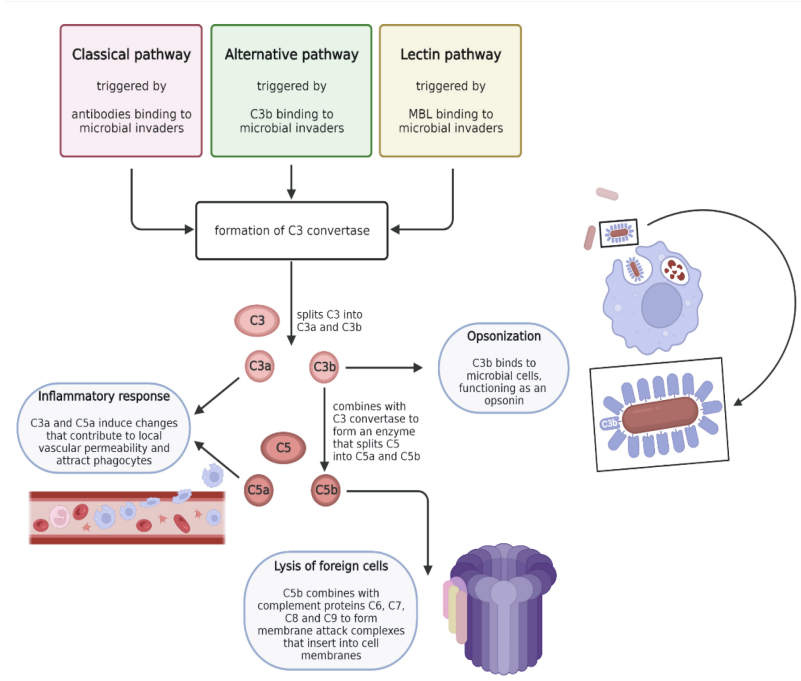


Figure 3. The role of the complement system in the inflammatory response to microbial invaders. Adapted from [30], created with BioRender.com

The compromised immune system

Genetic variability between hosts contributes to the risk of contracting IMD. In a large pediatric cohort, the susceptibility for meningococcal meningitis was found to be associated with single nucleotide polymorphisms in pathogen recognition receptor genes [33]. Genetic variations of toll-like receptors were recognized for their impact on susceptibility, severity and prognosis of meningococcal meningitis [34]. Patients suffering from IMD usually acquire the disease only once in life, except for individuals with complement deficiencies who are predisposed to recurrent meningococcal disease episodes [35]. Especially late complement component deficiency and other defects in components involved in the terminal complement pathway increase the risk of IMD [28]. The terminal pathway involves generation of a membrane-attack complex that can lead to dissipation of the membrane potential and eventually killing of the bacterium (Figure 3). An asplenic state, whether it is functional or anatomical asplenia, is another form of immunodeficiency that increases the risk of disease from encapsulated bacteria including meningococci, though pneumococcal infections are far more frequent in asplenia [36]. Therefore, it is recommended in many countries

including the Netherlands for both pediatric and adult asplenic patients to receive meningococcal and pneumococcal vaccinations during their lives [37]. Other host factors such as immunosuppressive or immunomodulating medication use may play a role in susceptibility to disease. Patients treated with monoclonal antibodies such as eculizumab – a terminal complement inhibitor – are at increased risk of IMD even after meningococcal vaccination [38, 39]. Therefore, the Advisory Committee on Immunization Practices advised to get immunized with meningococcal vaccinations before initiation of eculizumab treatment [40].

Meningococcal vaccines

Historically, the first meningococcal vaccines - developed since the 1970s - targeted the capsular polysaccharides. These plain polysaccharide vaccines provided a serogroup-specific anticapsular antibody response, but did not induce a long-term memory response and antibodies quickly waned. The induced immune response was T-cell independent, thus not inducing the production of memory B cells. Furthermore, immunogenicity was poor and short lasting when used in infants and toddlers [41]. Conjugate vaccines (polysaccharide conjugated to a carrier protein) that were developed about 20-25 years later markedly improved the prevention of IMD [42]. These vaccines have the advantage of inducing immunological memory and longer lasting protection as well as herd protection by reducing transmission [27]. With the induction of polysaccharide-specific antibodies following conjugate vaccination, a fast adaptive response after encountering the bacterium is established. Circulating antibodies provide immediate protection against invading meningococci and can prevent the rapid progression into severe disease [27]. Up until now, conjugate vaccination has been shown as the most effective strategy against IMD.

The capsule of MenB is similar to human fetal tissue, thus the MenB polysaccharide vaccine is poorly immunogenic and in addition is thought to potentially increase the risk of an auto-antibody response [43, 44]. Consequently, the MenB polysaccharide-based vaccine was never brought to the market. Another type of meningococcal vaccination was developed by use of reversed vaccinology that predicts possible vaccine candidates [45]. The immune response generated by currently licensed protein-based MenB vaccines is against one (rLP2086, Trumenba®) or four (4CMenB, Bexsero®) outer membrane antigens. Both vaccines contain protein(s) from the factor H-binding protein (fHbp) – Trumenba contains two subvariants of fHbp – while the composition of 4CMenB also includes, in addition to fHbp, three other antigens; Neisserial Heparin Binding Antigen (NHBA), Neisserial adhesin A (NadA) and an outer membrane vesicle

expressing PorA [46]. Hence, the protection provided by these vaccines is not limited to serogroup B but may extend to other serogroups as well.

Nowadays, many mono-, bi-and tetravalent polysaccharide and conjugate vaccines are licensed [47] and pentavalent vaccines (ACWYB, ACWYX) are under development and have shown promising results in phase 2 clinical trials [48-50].

Correlate of protection

While conjugate vaccines strictly induce the production of polysaccharide-specific antibodies, infection (carriage) may induce production of a variety of antibodies that can bind other outer surface antigens as well. Intramuscular immunization induces mainly serum antibodies, and the level of serum antibodies is used to evaluate immunogenicity of a meningococcal vaccine. The fluorescent bead-based multiplex immunoassay (MIA) is one of the laboratory assays that measures antigen-specific antibody concentrations, such as polysaccharide-specific serum IgG antibodies. Yet, this assay does not take into account the functionality of antibodies. Furthermore, antibodies against antigens such as outer membrane proteins - that may be acquired through carriage – can add to bactericidal activity of the serum.

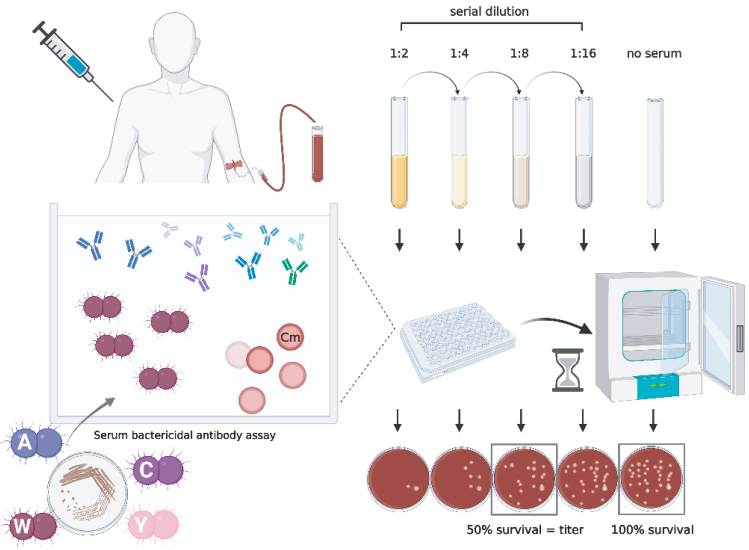


Figure 4. A graphical description of the serum bactericidal antibody (SBA) assay to determine the protection against meningococcal serogroup A, C, W and Y, as induced by complement (Cm)-mediated killing. The bacterial suspension and complement are added to serially two-fold prediluted serum and this mixture is incubated for one hour to determine the bactericidal titer. Created with BioRender.com.

Functional antibodies can be measured with the serum bactericidal antibody (SBA) assay (Figure 4) that determines the ability of human serum in different dilutions to kill meningococci in presence of an exogenous complement source (human or baby rabbit) [51]. The bactericidal titer is defined as the dilution that induces ≥50% killing of a target strain in presence of complement during incubation at 37 °C. This standardized and validated assay is internationally accepted as serological correlate of protection, with a titer of ≥8 as protective level and a titer of ≥128 indicating long-lasting protection[4, 52, 53].

Meningococcal disease and vaccination programme in the Netherlands

In 2002, a mass campaign for all individuals 1-18 years of age took place to limit the IMD-C outbreak that was ongoing since 1999–2000 (Figure 5). In addition to this campaign, a MenC conjugate vaccination (see: meningococcal vaccines) was introduced in the national immunization programme (NIP) for all children at 14 months of age in the Netherlands [54]. In the immediate years after the mass campaign, a steep decrease of cases was observed in vaccinated as well as in unvaccinated groups suggesting herd protection [16]. With IMD-C virtually absent in the years after 2002 and IMD-B steadily declining over time, a small rise in incidence of IMD-Y was observed in 2015-16 (Figure 5).

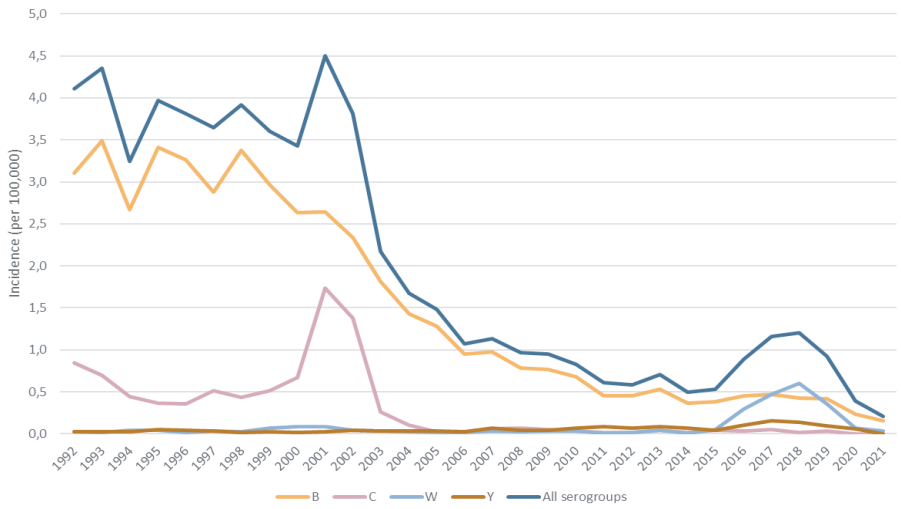


Figure 5. Incidence of invasive meningococcal disease by serogroup in the Netherlands, 1992-2021. (Source: RIVM)

More importantly, IMD-W emerged and incidence rates steeply increased from 2015 onwards. During the IMD-W outbreak, teenagers were disproportionally affected and mortality rates up to 24% were observed in this age group. Consequently, the meningococcal serogroup C conjugated to tetanus toxoid (MenC-TT) vaccine was replaced with a MenACWY-TT vaccine at age 14 months and an adolescent booster vaccination at the age of 14 years was introduced in the NIP [55]. Importantly, all children 14-18 year-olds were eligible to receive a single vaccination during the catch-up campaign between 2018-2019 to contain the IMD-W outbreak. This catch-up strategy aimed to limit transmission and induce herd protection while also directly protecting teenagers from disease. Although the first beneficial effects of this campaign could be observed early 2020, the COVID-19 control measurements in 2020-2021 resulted in a pronounced decline in all invasive bacterial infections including IMD in the Netherlands [56]. Ongoing surveillance is necessary to find whether a rebound effect of increased transmission will occur now the implementation of non-pharmaceutical interventions against COVID-19 have stopped.

Objectives and outline

The sudden emergence of IMD-W in the Netherlands highlights the changing epidemiology of the meningococcus and the importance of continuous surveillance. Emerging meningococcal infections may require adaptation of vaccination strategies for primary prevention. Knowledge on protective antibody levels with and without prior vaccination across different (age) groups in the population (serosurveillance) is essential to decide how to best intervene during an outbreak. The studies described in this thesis contribute to insights in the optimization of the protection against meningococcal ACWY disease in the Netherlands. The objectives were to assess protection after meningococcal conjugate vaccination in terms of evaluation of antibody responses, in different age groups and with different health status. These objectives lead to the following specific research questions:

- What is the current level of seroprotection against meningococcal disease nationwide in all age groups and what is the impact of the MenACWY vaccination on disease rates in the Netherlands?
- Is the antibody response to a MenACWY vaccination different in specific groups of the population (according to age, sex, and health status)?

In **chapter 2**, we investigated the naturally-and vaccine-induced seroprevalence among the population in the Netherlands. We evaluated antibody levels in almost 7,000 individuals aged 0-89 years in a cross-sectional survey pre-MenACWY implemen-

tation in 2016–17 and additionally, in longitudinal samples (subset) from a serosurvey that was performed because of the COVID-19 outbreak in 2020.

In **chapter 3,** we evaluated the vaccine impact and effectiveness of the MenACWY vaccination campaign in toddlers and teenagers during the IMD-W outbreak. IMD cases were extracted from the national surveillance system and age group-specific incidence rates before and after MenACWY vaccination implementation in 2018–19 were compared.

In **chapter 4**, we explored the long-term protection after a MenACWY vaccination in both adolescents and (middle-aged) adults. Waning of antibodies during five years postvaccination was modelled to predict the duration of seroprotection for the different age groups.

In **chapter 5**, the sex-related differences in the vaccine response during adolescence were examined. The immune response to vaccination may differ according to sex and differences may become more pronounced in adolescents due to hormonal differences. This may have implications for vaccination strategies, especially since an adolescent meningococcal booster vaccination was implemented in the NIP in the Netherlands to improve protection in this age group that has relatively high risk for IMD.

In **chapter 6**, we determined the vaccine response in adolescents with juvenile idiopathic arthritis (JIA) or inflammatory bowel disease (IBD) 14-18 years of age who received a MenACWY vaccination during the MenACWY campaign. The use of immunosuppressive medication is crucial in the treatment of these diseases, but might reduce the immune response to meningococcal vaccination. Yet, optimal protection against IMD is part of high-quality health care and needed in these – already vulnerable – patients. Evaluating immunogenicity after vaccination enables improvement of guidelines for patients with a compromised immune system.

In **chapter 7**, we described the immune response to a MenACWY vaccination in a heterogenous group of adolescents with primary or secondary immunodeficiencies.

The implications of the main findings as well as considerations to improve the protection against IMD by optimizing immunization programmes are discussed in **chapter 8**.

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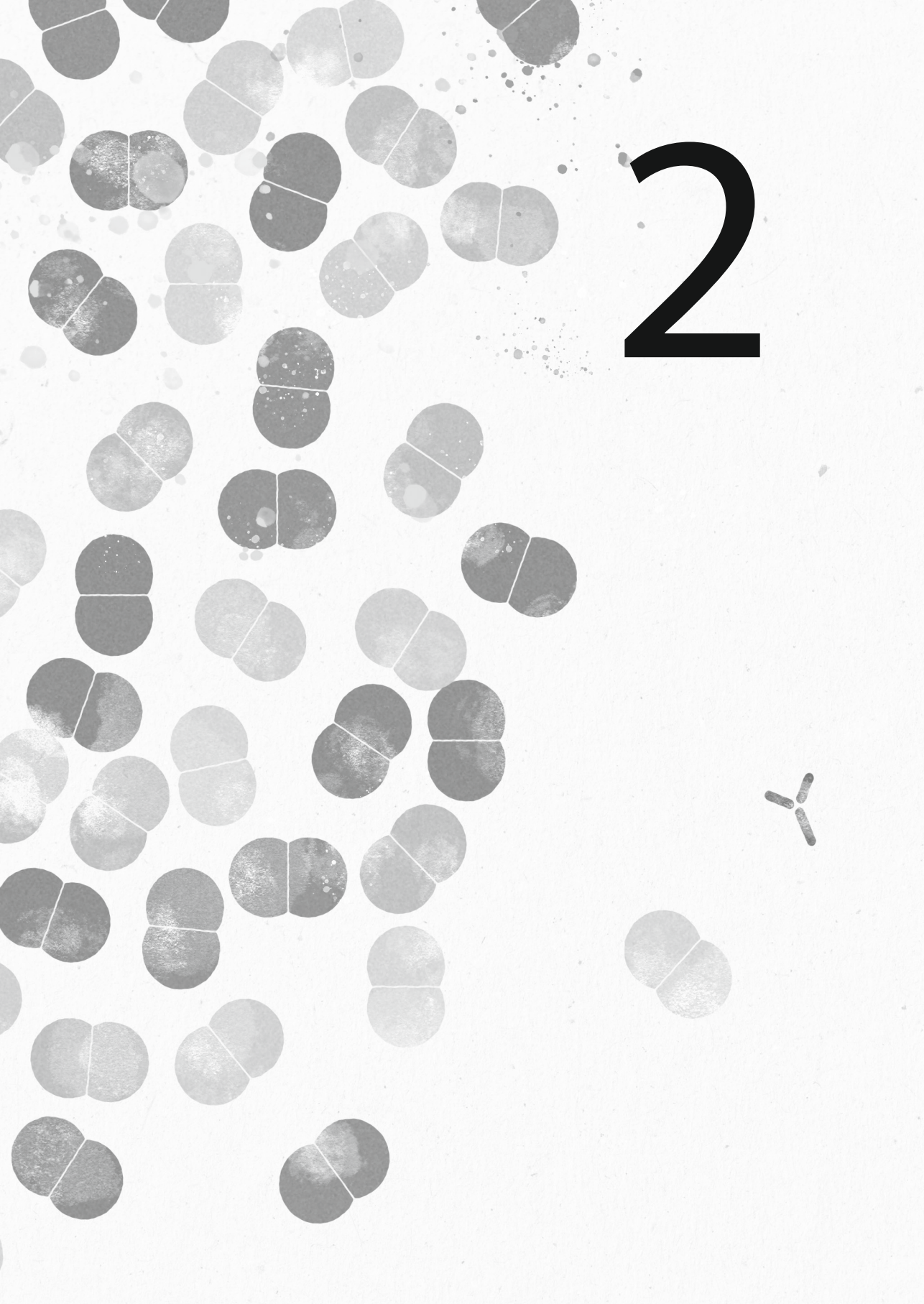

Seroprevalence of meningococcal ACWY antibodies across the population in the Netherlands: two consecutive surveys in 2016-17 and 2020

Milou Ohm[1], Mirjam J Knol[1], Eric R A Vos[1], Marjan J M Bogaard[1], Debbie M van Rooijen[1], Elisabeth A M Sanders[1], Hester E de Melker[1], Fiona R M van der Klis[1], Guy A M Berbers[1]

[1.] Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands

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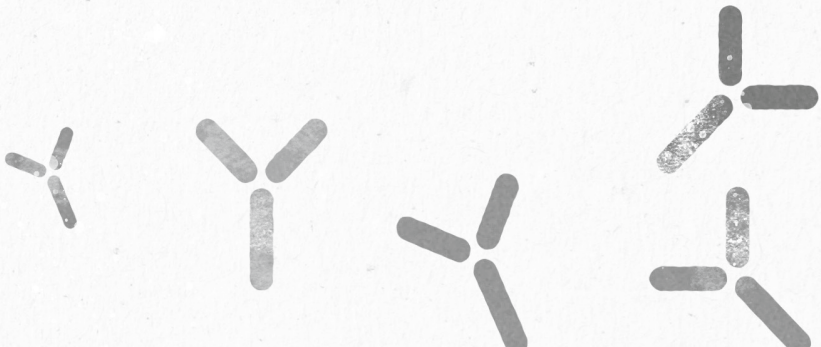

ABSTRACT

Background Meningococcal serogroup C (MenC) vaccination was introduced for 14-month-olds in the Netherlands in 2002, alongside a mass campaign for 1-18 year-olds. Due to an outbreak of serogroup W disease, MenC vaccination was replaced for MenACWY vaccination in 2018, in addition to introduction of a booster at 14 years of age and a catch-up campaign for 14-18 year-olds. We assessed meningococcal ACWY antibodies across the Dutch population in 2016-17 and 2020.

Methods In a nationwide cross-sectional serosurvey in 2016-17, sera from participants aged 0-89 years (n=6886) were tested for MenACWY-polysaccharide-specific (PS) serum IgG concentrations, and functional MenACWY antibody titers were determined in subsets. Moreover, longitudinal samples collected in 2020 (n=1782) were measured for MenACWY-PS serum IgG concentrations.

Results MenC antibody levels were low, except in recently vaccinated 14-23 month-olds and individuals who were vaccinated as teenagers in 2002, with seroprevalence of 59% and 20-46%, respectively. Meningococcal AWY antibody levels were overall low both in 2016-17 and in 2020. Naturally-acquired MenW immunity was limited in 2020 despite the recent serogroup W outbreak.

Conclusions This study demonstrates waning of MenC immunity 15 years after a mass campaign in the Netherlands. Furthermore, it highlights the lack of meningococcal AWY immunity across the population and underlines the importance of the recently introduced MenACWY (booster) vaccination.

INTRODUCTION

Invasive meningococcal disease (IMD) is a rare but severe disease that can lead to septicemia or meningitis [1]. The infectious cause is *Neisseria meningitidis*, a Gram-negative bacterium that is selective for the human host [2]. The bacterium is often carried asymptomatically in the nasopharynx, with highest carriage rates up to 24% observed in teenagers [3], while disease incidence is highest among infants [4].

In 1999-2001, a rapid increase in IMD incidence in the Netherlands was caused by the meningococcal serogroup C (MenC) bacterium [5]. Consequently, a MenC capsular polysaccharide conjugated to tetanus toxoid vaccine (MenC-TT) was introduced in the national immunization programme (NIP) for 14-month-olds in 2002. Additionally, a nationwide mass campaign for 1-18 year-olds (born between June 1983 and June 2001) took place that same year to restrain the epidemic with an estimated coverage of 94% [5]. In the years thereafter, a rapid decline in IMD serogroup C (IMD-C) cases was observed not only in those vaccinated, but also in unvaccinated cohorts [6]. Only five vaccine failures were reported since the implementation of the vaccine, of which two patients suffered from underlying immune deficiency [7]. In 2015-16, there was a rise in IMD serogroup W (IMD-W) incidence and most cases were caused by a hyperinvasive strain that belongs to a single sequence type 11 clonal complex (cc11) [8]. This strain circulated in nearby countries such as in the United Kingdom as well [9]. During the IMD-W epidemic, unusual high mortality rates were observed across all ages, but especially in 14-24 year-olds with an overall case fatality rate of 26% in this age group [7]. Hence, the MenC-TT vaccine for 14-month-olds was replaced by a MenACWY-TT vaccine in 2018 and a MenACWY booster vaccination at the age of 14 years was implemented in the NIP in 2020. Additionally, a catch-up campaign targeting adolescents born between January 2001 and December 2005 (i.e., 14-18 year-olds) was executed in 2018-19 with an uptake of 84% [10]. The MenACWY vaccination programme was very effective in the target population and also a reduction in IMD-W incidence was observed in vaccine-noneligible groups [11].

Due to the rapidly evolving nature of IMD, immediate host defense mechanisms causing bacterial killing are vital in the protective response. Bactericidal antibodies, together with the complement system, can provide this fast mode of immune action [12]. Those antibodies may be acquired naturally through infection, or induced by vaccination, and can be present in serum as well as in mucosal tissues [13]. Most meningococcal vaccinations induce the production of polysaccharide-specific (PS)

antibodies targeting the capsule of the bacterium – while newer vaccines that are developed to protect against MenB include proteins from other surface structures [12].

Serosurveillance studies not only provide valuable knowledge on protection throughout the population, but also enable the detection of changes in protection levels when conducted periodically and support optimization of national vaccination policy. Our objective was to assess the meningococcal serological status in two consecutive cross-sectional population-based surveys recently conducted in the Netherlands [14, 15]. We determined meningococcal ACWY antibody levels in sera collected in 2016-17, thereby investigating both vaccine-induced MenC, and naturally-acquired MenAWY immunity. Furthermore, from a subset of these participants, longitudinal sera were available from a 2020-serosurvey – initially collected in response to the Coronavirus disease 2019 (COVID-19) pandemic – and we explored the post IMD-W epidemic level of MenW IgG serum antibodies in these sera.

METHODS

Study design

The third cross-sectional population-based serosurveillance study in the Netherlands, the PIENTER3-study (clinical trial number NL5467), was conducted between February 2016 and October 2017. Details of the survey methods, data collection and inclusion have been described previously [14]. Briefly, a randomly-selected national sample (NS) of participants 0-89 years of age was drawn from the population registry using a two-stage cluster technique (i.e., forty municipalities within five regions proportional to size), applying age-stratified sampling within each included municipality. An additional sample of participants living in areas with a low vaccination coverage (LVC) was also included in this study. All participants in this study (n=6886) were asked to fill in a questionnaire including questions on vaccination history and to provide a blood sample. Participants from whom there was no available serum for laboratory analyses (dry blood spots, n=208; missing, n=5) were excluded. Written informed consent was obtained from all participants aged 12 years and older and from both parents or legal guardians when a subject was under the age of 16 years at enrolment. The study was conducted in accordance with ethical guidelines (the Declaration of Helsinki) and the study proposal was ethically approved by the Medical Ethics Committee Noord-Holland (METC number: M015-022).

Participants from the PIENTER-3 study who provided consent to be approached in a follow-up study were consulted to take part in a prospective serosurvey that aimed to monitor nationwide SARS-CoV-2 seroprevalence in the population of the Netherlands following the COVID-19 pandemic (PIENTER-corona [PICO]-study, clinical trial number NL8473) [16]. This serosurvey started in 2020 and encompasses several rounds of blood collection. For the current study, 1782 participants (all formally belonging to the PIENTER3 NS-sample and excluding SARS-CoV-2 positive participants and those with a low serum volume) were included from the June-July 2020 PICO-round [15] to explore MenW immunity at the end of the IMD-W epidemic and after implementation of MenACWY vaccination. Participants provided a self-collected finger-stick blood sample and filled out a questionnaire.

Serological analyses

Serum samples were stored at -80°C until analysis. Available sera were tested for MenA-, MenC-, MenW-, and MenY-PS-specific serum IgG concentrations, using a fluorescent-bead-based multiplex immunoassay (MIA) [17-19]. For all four serogroups, a value of 0.01 µg/mL was assigned to sera that fell below the lower limit of quantitation. Since 2019, a protein-free buffer (Surmodics) is used in the meningococcal MIA at our laboratory, and the data from both surveys (2016-17 and 2020) as described here were generated with this buffer. We executed bridging experiments for the serological analyses to ensure consistency over time, for the MIA as well as for the serum bactericidal antibody (SBA) assay as described below.

Functional antibodies were assessed in the 2016-17-survey with the serum bactericidal antibody (rSBA) assay using baby rabbit complement (Pelfreez, lot 22841) and MenA strain 3125, MenC strain C11 [20], MenW strain MP01240070 and MenY strain S-1975 as target strains. The dilution of the sera that yielded ≥50% bacterial killing after 60 minutes incubation was defined as the serum bactericidal titer, and the internationally accepted correlate of protection of rSBA titer ≥8 was used for analyses [21-23]. A value of 2 was assigned when rSBA titers fell below the cut-off of the assay (titer below 4). All sera that were tested with the SBA assay were selected from the NS. A large subset of n=1041 sera within stratified age-bands was randomly selected (blinded to the IgG results) to determine the MenC rSBA titer. For MenW and MenY, which are both endemic but were non-vaccine serogroups in 2016-17, we aimed to investigate naturally-acquired MenWY functional antibodies and their corresponding PS-specific IgG. A non-random subset of samples (n=155 for both serogroups) with either a low IgG concentration (<1 µg/mL) or a high IgG concentration (≥2 µg/mL) was selected for the rSBA assay. The selected subsets for MenW and MenY consisted of sera from

participants across all ages. A random subset of n=167 samples was selected for the MenA rSBA assay (blinded to the IgG result) to detect any killing capacity against this serogroup, that has never been endemic in the Netherlands.

Statistical methods

All statistical analyses were performed using Excel, GraphPad Prism 8 and SPSS Statistics v24. Seroprevalence, rSBA geometric mean titers (GMTs) and geometric mean IgG concentrations (GMCs) were calculated with corresponding 95% confidence intervals (CIs) in the 2016-17-survey. GMCs were weighted proportionally to the reference population (Dutch population, 1 January 2017) with sex, age, ethnic origin and urbanization degree taken into account for both the NS and the LVC sample. Also, the survey design (with regions as strata and municipalities as clusters) was accounted for in the analyses. Differences in weighted GMCs between the NS and LVC sample were determined on logarithmic (log)-transformed values with an independent samples t-test. Seroprevalence was expressed as the proportion of participants with an rSBA titer ≥8 and corresponding 95% CIs were calculated using Wilson-Brown with continuity correction. Seroprevalence was compared per age group to the seroprevalence in the second serosurveillance study in 2006-07 (PIENTER2-study) that was described previously [24], and differences were determined with a Fisher's exact test. Statistical tests were two-sided. A p-value below 0.05 was considered statistically significant.

In the 2020-survey, we calculated unweighted GMCs with corresponding 95% CI per age groups as used in the 2016-17-survey. Furthermore, we tried to determine how many participants were infected during the IMD-W epidemic. Since information on meningococcal vaccination history was not obtained due to the nature of questionnaire, we assigned an infection/vaccination status to each participant based on their individual IgG concentrations, with use of an arbitrary cut-off of ≥0.1 µg/mL. Sera with IgG concentrations for three or all four serogroups ≥0.1 µg/mL were labelled as 'probable recent MenACWY vaccination'. When MenW IgG was ≥0.1 µg/mL, together with a 10-fold rise in MenW IgG concentration compared to the corresponding MenW IgG concentration in the 2016-17 sample, the label 'probable recent MenW infection' was assigned. If the MenW IgG was ≥0.1 µg/mL but lower in the 2020-survey than in the 2016-17-survey, the sample was labelled as 'other', and if results were contradictory or inconsistent between serogroups or timepoints, the label 'unknown' was assigned.

RESULTS

Study and participant characteristics

We included 5552 sera from the NS and 1334 sera from the LVC sample (in total 6886 sera) from the 2016-17-survey. Around ten percent more females (54.3% and 55.9% respectively) than males (45.7% and 44.1% respectively) were included for analyses in both the NS and LVC sample (Table 1). From the 2020-survey, we included 1782 samples for analyses. In general, the sociodemographic characteristics in the 2020 sample (Supplementary Table 1) were similar compared to the NS in 2016-17, albeit with a slight difference in age distribution between the surveys, more participants with a Dutch ethnic background (78.4% versus 89.4%) and more highly educated participants (38.6% versus 50.1%) in the 2020 sample compared to the 2016-17 sample.

Table 1. Sociodemographic characteristics of participants with a serum sample available for MenACWY IgG antibody measurement from the national sample (n=5552) and the low vaccination coverage (LVC) sample (n=1334) in the 2016-17-survey

	National sample N=5552	LVC sample N=1334
Age groups (years)	**N (%)**	**N (%)**
0-14	1384 (24.9%)	367 (27.5%)
15-24	671 (12.1%)	195 (14.6%)
25-34	730 (13.1%)	190 (14.2%)
35-44	653 (11.8%)	162 (12.1%)
45-64	1256 (22.6%)	258 (19.3%)
65-90	859 (15.5%)	162 (12.1%)
Sex		
Male	2535 (45.7%)	588 (44.1%)
Female	3017 (54.3%)	746 (55.9%)
Educational level*		
High	2015 (38.6%)	295 (23.4%)
Middle	1790 (34.3%)	529 (41.9%)
Low	1413 (27.1%)	437 (34.7%)
Urbanization degree		
Highly urbanized	1168 (21.0%)	0 (0%)
Urbanized	1815 (32.7%)	0 (0%)
Moderate urbanized	1064 (19.2%)	156 (11.7%)
Little urbanized	1016 (18.3%)	743 (55.7%)
Countryside	489 (8.8%)	435 (32.6%)
Ethnic background		
Dutch	4353 (78.4%)	1279 (95.9%)
other-Western	365 (6.6%)	32 (2.4%)
non-Western	834 (15.0%)	22 (1.7%)

Table 1. *(Continued)*

	National sample N=5552	LVC sample N=1334
MenC vaccination coverage in eligible cohorts**	**vaccinated/total (%)**	**vaccinated/total (%)**
14-23 month-olds	72/78 (92.3%)	10/17 (58.8%)
2-year-olds	64/68 (94.1%)	9/17 (52.9%)
3-year-olds	67/69 (97.1%)	17/23 (73.9%)
4-year-olds	85/92 (92.4%)	12/19 (63.2%)
5-9 year-olds	302/320 (94.3%)	52/75 (69.3%)
10-14 year-olds	332/338 (98.2%)	57/79 (72.2%)
15-19 year-olds	240/271 (88.6%)	57/78 (73.1%)
20-24 year-olds	277/400 (69.3%)	57/117 (48.7%)
25-29 year-olds	52/366 (14.2%)	7/96 (7.3%)
30-34 year-olds	27/363 (7.4%)	8/94 (8.5%)

*percentages calculated on the total number of participants with information on educational level available (missing for 334 participants from the national sample and 73 participants from the LVC sample);
** self-reported vaccination status according to questionnaire

MenC SBA seroprevalence in the national sample in 2016-17

A change in the proportion of MenC-protected individuals (rSBA titer ≥8) was observed in 2016-17 compared to 2006-07 (Figure 1). Especially in toddlers, the seroprevalence was significantly (p=0.009) lower in 2016-17 with 59% (95% CI 40.8-75.8%) of 14-23-month-olds protected compared to 90% previously (in both surveys the mean age was 18 months) (Table 2).

Table 2. Meningococcal C (MenC) geometric mean titers (GMTs) and seroprevalence (rSBA titer ≥8) per age group in the national sample in 2016-17 and MenC seroprevalence per age group in the national sample in 2006-07.

		2016-17		2006-07	
Age groups (years)	**GMT (95%CI)**	**No of samples**	**Seroprevalence (95%CI*)**	**No of samples**	**Seroprevalence (95%CI)**
0–7 months	2 (NA)	30	0% (NA)	59	0% (NA)
8–13 months	2.45 (1.62–3.69)	31	3.2% (0-18.5)	62	3.3% (0-8)
14–23 months	39.74 (15.16–104.16)	32	59.4% (40.8-75.8)	27	89.7% (79-100)
2	4.84 (2.38–9.86)	29	20.7% (8.7-40.3)	42	61.9% (49.2-74.6)
3–4	2.30 (1.96–2.69)	45	4.4% (1-16.3)	106	36.8% (26.4-47.2)
5–6	2.83 (2.00–3.99)	42	9.5% (3.1-23.5)	49	42.9% (29.8-55.9)
7–8	3.14 (1.98–4.96)	34	11.8% (3.8-28.4)	56	39.3% (27.8-50.7)
9–10	3.03 (2.01–4.58)	40	10.0% (3.3-24.6)	73	49.3% (34.6-64)
11–12	2.90 (2.02–4.16)	43	9.3% (3-23.1)	72	73.6% (65-82.2)

Table 2. *(Continued)*

Age groups (years)	GMT (95%CI)	2016-17		2006-07	
		No of samples	Seroprevalence (95%CI*)	No of samples	Seroprevalence (95%CI)
13–14	2.74 (1.86–4.04)	44	6.8% (1.8-19.7)	65	80% (70.5-89.5)
15–16	3.69 (2.15–6.34)	43	11.6% (4.4-25.9)	55	83.6% (74.4-92.8)
17–18	2.51 (1.88–3.33)	40	7.5% (2-21.5)	43	93% (86.2-99.9)
19–21	5.31 (3.00–9.41)	61	19.7% (11-32.2)	88	96.6% (92.5-100)
22–25	5.93 (3.71–9.47)	80	26.3% (17.3-37.5)	104	59.6% (50.9-68.4)
26–30	14.45 (8.99–23.23)	106	46.2% (36.6-56.1)	69	33.3% (21.2-45.5)
31–39	5.21 (3.59–7.57)	117	20.5% (13.8-29.2)	58	37.9% (18.5-57.3)
40–49	3.17 (2.03–4.98)	42	9.5% (3.1-23.5)	49	26.5% (14.3-38.7)
50–59	2.55 (1.87–3.46)	46	6.5% (1.7-19)	52	36.5% (19.8-53.3)
60–69	2.66 (1.90–3.71)	44	6.8% (1.8-19.8)	54	29.6% (20.4-38.9)
70–79	2.89 (1.96–4.25)	51	7.8% (2.5-19.8)	37	35.1% (15.8-54.5)
80+	2.07 (1.97–2.17)	41	0% (NA)	0	NA
Total	4.22 (3.78-4.70)	1041	16.8% (14.6-19.3)	1220	50.5% (47.1-53.9)

Abbreviations: CI, confidence interval; NA, not applicable. *determined with Wilson continuity correction.

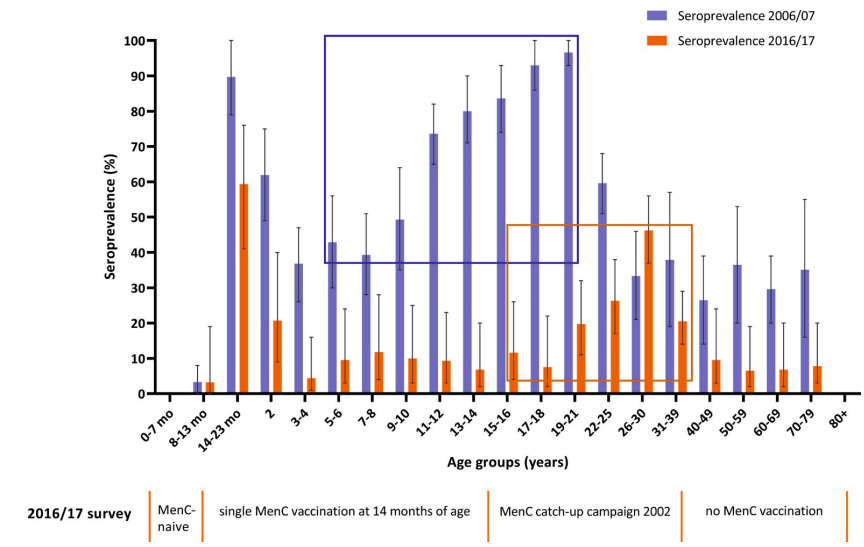

Figure 1. Meningococcal serogroup C (MenC) seroprevalence determined by rSBA assay (titer ≥ 8 defined as seroprotected) in the national sample per age group with 95% confidence intervals in 2006-07 and 2016-17.

Purple (left) box represents age groups with MenC vaccine-eligible (for the mass campaign in 2002) individuals in 2006/07. Orange (right) box represents age groups consisting (partly)* of MenC vaccine-eligible (for the mass campaign in 2002) individuals in 2016/17. *the age group of individuals aged 31–39 years also contains vaccine-noneligible individuals. Abbreviations: mo, months.

The protected proportion rapidly decreased with age in the 2016-17-survey. On average, only 10% of children aged 3-18 years showed rSBA titers ≥8. Low seroprevalence was also observed in unvaccinated adults, with only 6.5-9.5% of individuals aged 40-80 years showing bactericidal activity, while ten years prior 26-36% of adults in these age groups showed a protective rSBA titer. A substantial part of young adults aged 19-30 years was protected in 2016-17, with a seroprevalence gradually increasing from 20% in 19-21 year-olds to 46% in 26-30 year-olds (Table 2), reflective of vaccine-induced humoral immunity as they were vaccine-eligible (at an age of 3-16 years) during the MenC mass campaign 15 years prior.

MenACWY-PS-specific IgG concentrations in the national sample in 2016-17

The highest MenC-PS-specific GMC among children was observed in 1- and 2-year-olds with GMCs of 0.68 and 0.93 µg/mL, respectively (Figure 2). The MenC-PS-specific GMC gradually declined in children from 2 to 4 years of age to 0.33 µg/mL. In children and teenagers aged between 5 and 19 years, low MenC-PS-specific GMCs around 0.20 µg/mL were observed. In adults, the highest GMCs were observed in 25-29 year-olds (0.86 µg/mL, 95%CI 0.61-1.20) and 30-34 year-olds (0.42 µg/mL, 95%CI 0.30-0.60). Those age groups comprised individuals who were eligible for vaccination 15 years prior to serum collection in this study, with a single MenC vaccination given at an age of 10-18 years.

In adults aged 35 years and older, the GMCs were low and ranged between 0.04-0.12 µg/ml, which was comparable to the 2006-07 survey (data not shown). Low MenA-, MenW- and MenY-PS-specific GMCs were found in children as well as in adults (Figure 2), reflecting the lack of IgG antibodies in a population that was not MenAWY-immunized at time of the 2016-17-survey. As observed in the 2006-07 survey [24], a minor but steady increase of MenA-PS-specific GMC was observed with age, however this cannot be explained by natural immunity since IMD-A is not prevalent in the Netherlands, but rather by cross-reactive antibodies that also recognize the capsule of *Bacillus pumilus* [25].

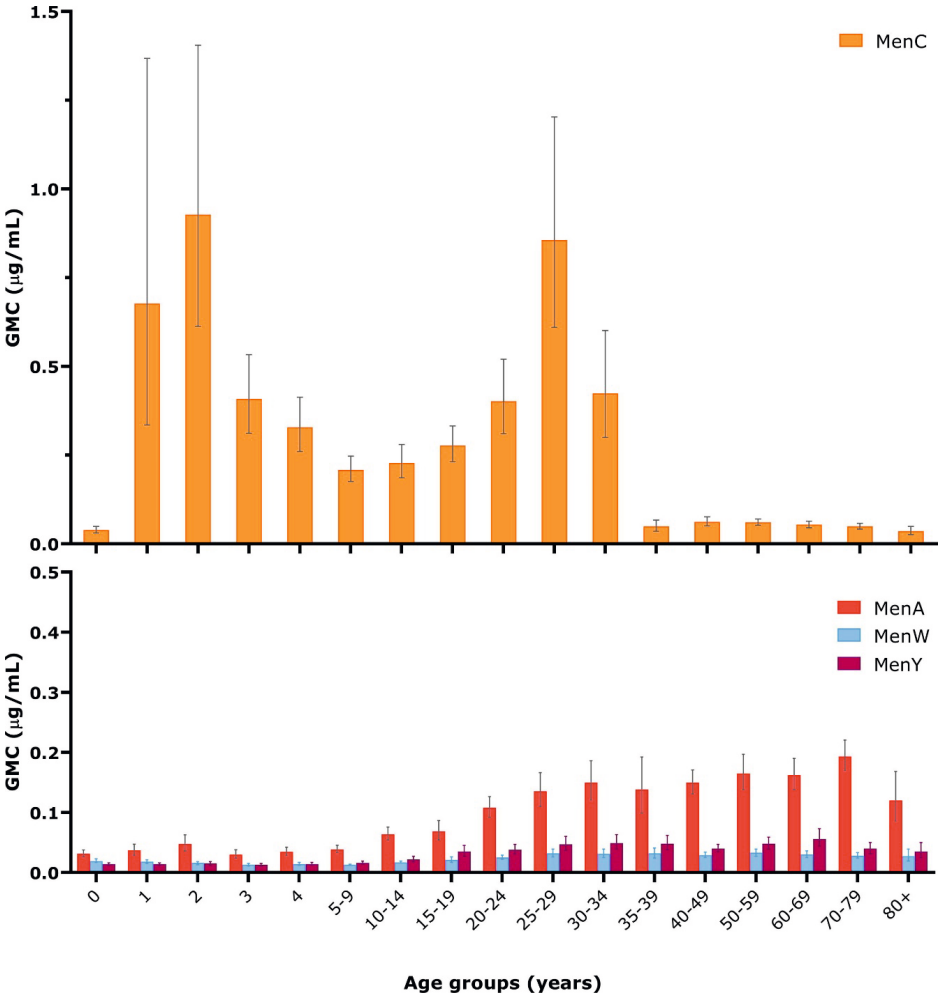

Figure 2. Meningococcal ACWY polysaccharide (MenACWY-PS)-specific weighted geometric mean concentrations (GMCs) with 95% confidence intervals (CI) in the general Dutch population (i.e., the national sample) in 2016-17.

MenC-PS-specific IgG concentrations in the LVC sample in 2016-17

We found that only 58.8% of 14-23 month-olds in the LVC sample reported a history of MenC vaccination, in contrast to 92.3% in the NS (Table 1). Weighted MenC GMCs were significantly lower in the LVC sample than in the NS for 1-, 2- and 25-29 year-olds (Figure 3). In the other age groups until the age of 30 years, slight differences between weighted GMCs were observed, albeit not significant. No differences between the NS and LVC sample were observed in unvaccinated cohorts of adults 31 years and older.

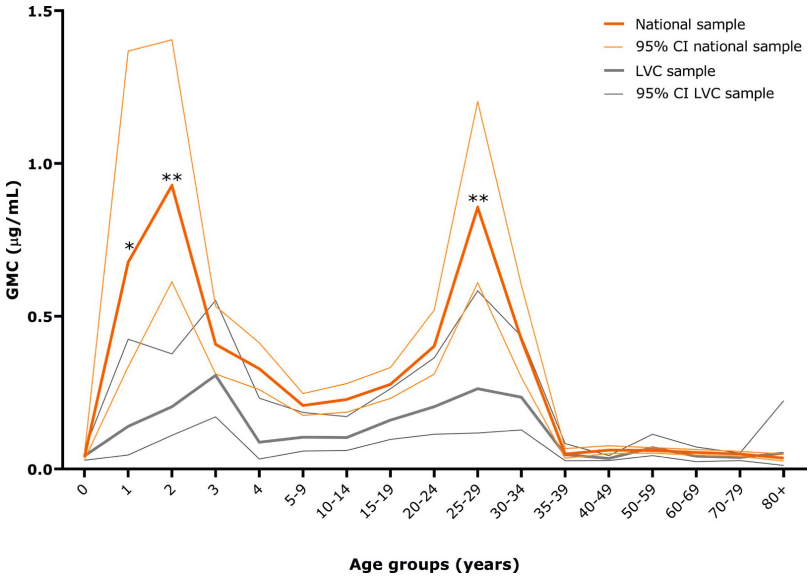

Figure 3. Meningococcal C polysaccharide (MenC-PS)-specific weighted geometric mean concentrations (GMCs) with 95% confidence intervals (CIs) in the general Dutch population (i.e., the national sample) compared to the low vaccination coverage areas (LVC) in 2016-17.
* p-value < 0.05, ** p-value < 0.001.

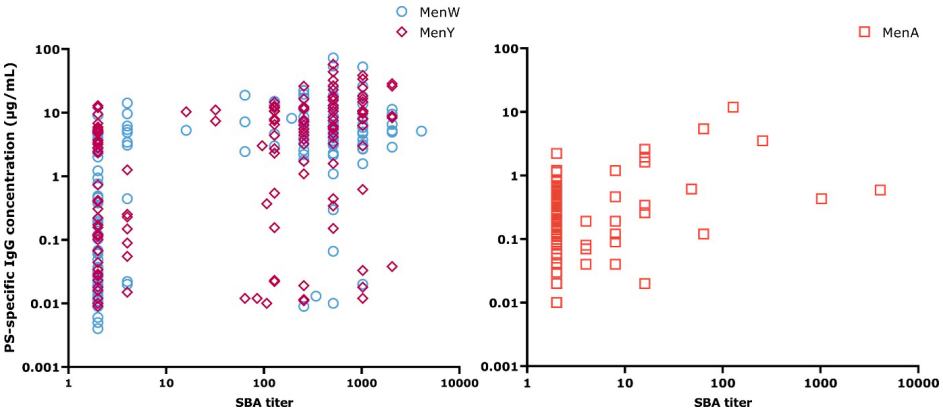

Figure 4. Meningococcal W and Y (left panel) and A (right panel) rSBA titers and corresponding polysaccharide (PS)-specific IgG concentrations (µg/mL) in a subset of sera from the national sample in 2016-17.

MenAWY serum bactericidal activity in the national sample in 2016-17

For all three serogroups, a low rSBA titer (<8) corresponded with a wide range of PS-specific IgG concentrations, from 0.01 to more than 10 µg/mL (Figure 4). The majority of sera with bactericidal activity against a specific serogroup also showed a high corresponding PS-specific-IgG concentration. However, there were also 12 sera (7.7%) with a MenY-PS-specific IgG concentration below 0.1 µg/mL, while the rSBA titer for that serogroup was ≥8. This was also true for 5 sera (3.2%) tested for MenW, indicating that bactericidal activity in those cases was not IgG PS-specific. Only 19 of the 167 (11.3%) randomly selected sera that were tested for MenA showed bactericidal activity against this serogroup.

MenW-PS-specific IgG concentrations in 2020

The MenW-PS-specific GMC in the vaccine-eligible age group of 15-19 years was 0.8 µg/mL, while in all other age groups the GMC was below 0.1 µg/mL. Correspondingly, in the majority of samples (1445/1782, 81%) the MenW-PS-specific IgG concentration was below 0.1 µg/mL (Table 3). Almost two-third of the samples (215/337) with MenW IgG ≥ 0.1 µg/mL also had an IgG ≥0.1 µg/mL for two or three others serogroups, and were labelled as probably vaccinated recently or in the past with a MenACWY vaccine. Surprisingly, the age of those individuals ranged from 3 years to 81 years and less than half was vaccine-eligible according to their age. Of the remaining 122 participants with MenW IgG ≥0.1 µg/mL, 85 participants showed a lower IgG concentration in the 2020-survey than in the 2016-17-survey. Only eight out of 1782 participants (0.45%) had a MenACWY IgG profile that suggested a natural infection with MenW in the years 2017-2020, and were aged between 19-59 years, of which five were aged 42-52 years.

Table 3. Individuals with meningococcal serogroup W polysaccharide (MenW-PS) specific IgG ≥ or <0.1 µg/mL in the 2020-serosurvey.

	Label based on MenACWY-PS-specific IgG profile*	N (%)
MenW ≥0.1 µg/mL	Natural infection	8 (0.45%)
	Probably MenACWY vaccinated	215 (12%)
	Other	96 (5%)
	Unknown	18 (1%)
MenW <0.1 µg/mL	No detectable infection or vaccination	1445 (81%)
	Total	1782

*sera with IgG concentrations for three or all four serogroups ≥0.1 µg/mL were labelled as 'probable recent MenACWY vaccination'. When MenW IgG was ≥0.1 µg/mL, together with a 10-fold rise in MenW IgG concentration compared to the corresponding MenW IgG concentration in the 2016-17 sample, the label 'probable recent MenW infection' was assigned. If the MenW IgG was ≥0.1 µg/mL but lower in the 2020-survey than in the 2016/17-survey, the sample was labelled as 'other' and if results were contradictory or inconsistent between serogroups or timepoints, the label 'unknown' was assigned.

DISCUSSION

Here, we assessed meningococcal ACWY IgG antibody concentrations and functional antibody titers in the Dutch population encompassing a 4-year period (2016-2020) in which a MenACWY vaccination was implemented. We found a lack of immunity against meningococcal serogroups ACWY across most age groups in 2016-17. Only recently vaccinated toddlers and individuals who were vaccinated at teen age during the MenC mass campaign in 2002 showed bactericidal MenC activity, albeit lower than in the cross-sectional serosurvey conducted ten years prior. These results emphasize the waning of MenC immunity across the population in 2016-17, 15 years after implementation of a MenC vaccination. Furthermore, we found MenW immunity in the 2020-survey in teenagers who were vaccine-eligible during the MenACWY catch-up campaign in 2018-19, but low MenACWY antibody levels in vaccine-noneligible age cohorts.

Individuals who were 12 years or older at time of MenC mass campaign in 2002 benefit most on an individual level 15 years postvaccination. Almost half of individuals aged 26-30 years (who were all vaccine-eligible in the mass campaign) showed bactericidal activity against MenC, and the GMT was higher than in any other adult age group. This indicates that vaccinating teenagers with a single dose as part of a mass campaign during an epidemic protects half of them far into adulthood. The previous survey took place in 2006-07, shortly after the IMD-C epidemic in the Netherlands, whereas circulation of MenC is now very limited and thus natural boosting virtually absent [6]. This was also illustrated by the low seroprevalence estimates that we found in older, unvaccinated age cohorts. Currently, IMD-C cases only sporadically occur in both vaccinated and unvaccinated persons [7]. However, we believe that the observed overall low seroprevalence could have led to lack of MenC herd immunity in the nearby future if the recently introduced teenage MenACWY booster vaccination had not been implemented in the NIP in response to a IMD-W epidemic [8].

Surprisingly, less than two-third of vaccine-eligible toddlers (14-23 month-olds) appeared protected shortly after the vaccination timepoint in the NIP. This is despite the fact that vaccination coverage was 92% in this group, which is in line with the national uptake [7]. In the previous survey that took place ten years before, higher SBA seroprevalence rates were detected in all young children, with levels up to 90% protection in toddlers [24]. Although we cannot rule out that the ten-year gap between the studies might have influenced the results, we executed extensive bridging experiments to detect relevant differences in the assays, and validated the consistency of

the assays over time. Our results are in line with a Portuguese serosurveillance study that was recently published which showed a seroprevalence of 15.5% in 2-4 year-olds, who were primed at the age of 12 months with a MenC conjugate vaccine [26]. In a survey carried out in the UK nine year postvaccination, 15.6% of children aged 11 years - who were vaccinated once in the second year of life - was seroprotected [27]. This is comparable to 10% protection in the Dutch 10-year-olds, who were also vaccinated in the second year of life. Although poor persistence of antibodies in MenC-vaccinated young children has been described [28, 29], we do not have an explanation for the relatively high proportion of toddlers in the current study who lacked functional antibodies shortly after vaccination. We expected to find higher seroprevalence levels based on previous immunogenicity and vaccine effectiveness studies (including the Dutch 2006-07-survey) that showed that short-term protection reached almost 100% in young children [30-33]. Nonetheless, this cohort is also protected indirectly through herd immunity [6], which is supported by a very low number of IMD-C cases in recent years in the Netherlands [7].

Outbreaks are extensively described for IMD, but mostly in students or military settings or other mass gatherings [34, 35] and not necessarily in geographic spots with low coverage, in contrast to other vaccine-preventable infectious diseases [36, 37]. In the LVC sample, the MenC GMC was significantly lower than in the NS sample, but no meningococcal outbreaks in LVC areas have been described up until now. This will probably be the case as long as circulation of meningococci is restricted, although higher vaccine uptake in LVC areas would provide better direct protection.

While vaccination may induce production of IgA/IgM antibodies in addition to PS-specific IgG, carriage may elicit a local immune response on top of that [38, 39]. Moreover, natural infection might lead to antibodies against surface antigens not included in the vaccine and add to bactericidal activity [38]. On the other hand, the observation of a high IgG concentration with a low corresponding SBA titer could be explained by the presence of low-avidity or non-functional antibodies. We found that some sera showed bactericidal activity against MenW or MenY, while the corresponding PS-specific IgG concentration was low. It has previously been described that the correlation between IgG concentrations and SBA titers was lower in unimmunized groups [40], possibly explained by immunity conferred differently by natural infection compared to vaccination. Only 0.45% of the participants in the 2020-survey showed a MenACWY IgG profile that suggested recent natural MenW infection, even though IMD-W incidence rates had substantially increased in the Netherlands [9]. However, a recent carriage prevalence study among college students in the Netherlands [41] showed 1.3% MenW

carriage during the epidemic in the fall of 2018 in this age group, that is known to show higher carriage rates compared to other age groups. Therefore, the percentage of infected participants that we found seemed reasonable but meningococcal PS-specific IgG concentrations alone in a serosurvey must be interpreted carefully, especially in unvaccinated groups, and particularly also when results are not weighted for baseline characteristics.

One of the strengths of this study is the population-based nature with sampling in a representative sample every ten years, thereby enabling the detection of changes in seroprevalence over the years. The additional 2020-survey including longitudinal samples enabled us to investigate antibody levels shortly after the IMD-W epidemic as well. However, meningococcal vaccination history was not obtained in this study. This complicated the distinction between vaccination and natural infection and we had to use the MenACWY IgG profile for the exploration, which could have led to misclassification. Furthermore, SBA measurements would have been useful to support the IgG results. However, only small volumes of sera were collected in the 2020-survey, thereby precluding functional antibody measurements necessitating larger amounts of sera. Since we had to exclude participants that were either tested SARS-CoV-2 positive or had a very low serum volume (<50 µl), it must be taken into account that the representativeness of the sample might have been affected.

To conclude, we found that the majority of both children and adults in the Netherlands are currently poorly protected individually against MenACWY and we showed waning of MenC immunity that was induced by the mass campaign in 2002. Moreover, these consecutive studies underline the importance of the teenager MenACWY booster vaccination that was recently implemented in the NIP as this will improve the duration of seroprotection in this cohort and might provide indirect protection for cohorts with low antibody levels.

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Supplementary Table 1. Sociodemographic characteristics of participants with a serum sample available for MenACWY IgG antibody measurement in the 2020-survey

Age groups, years	**N (%)**
3-14	265 (14.9%)
15-24	157 (8.8%)
25-34	235 (13.2%)
35-44	264 (14.8%)
45-64	520 (29.2%)
65-90	341 (19.1%)
Sex	
Male	778 (43.7%)
Female	1004 (56.3%)
Educational level*	
High	880 (50.1)
Middle	588 (33.4)
Low	290 (16.5)
Urbanization degree	
Highly urbanized	326 (18.3)
Urbanized	559 (31.4)
Moderate urbanized	414 (23.2)
Little urbanized	316 (17.7)
Countryside	167 (9.4)
Ethnic background	
Dutch	1593 (89.4)
other-Western	94 (5.3)
non-Western	95 (5.3)

*percentages calculated on the total number of participants with information on educational level available (missing for 24 participants)

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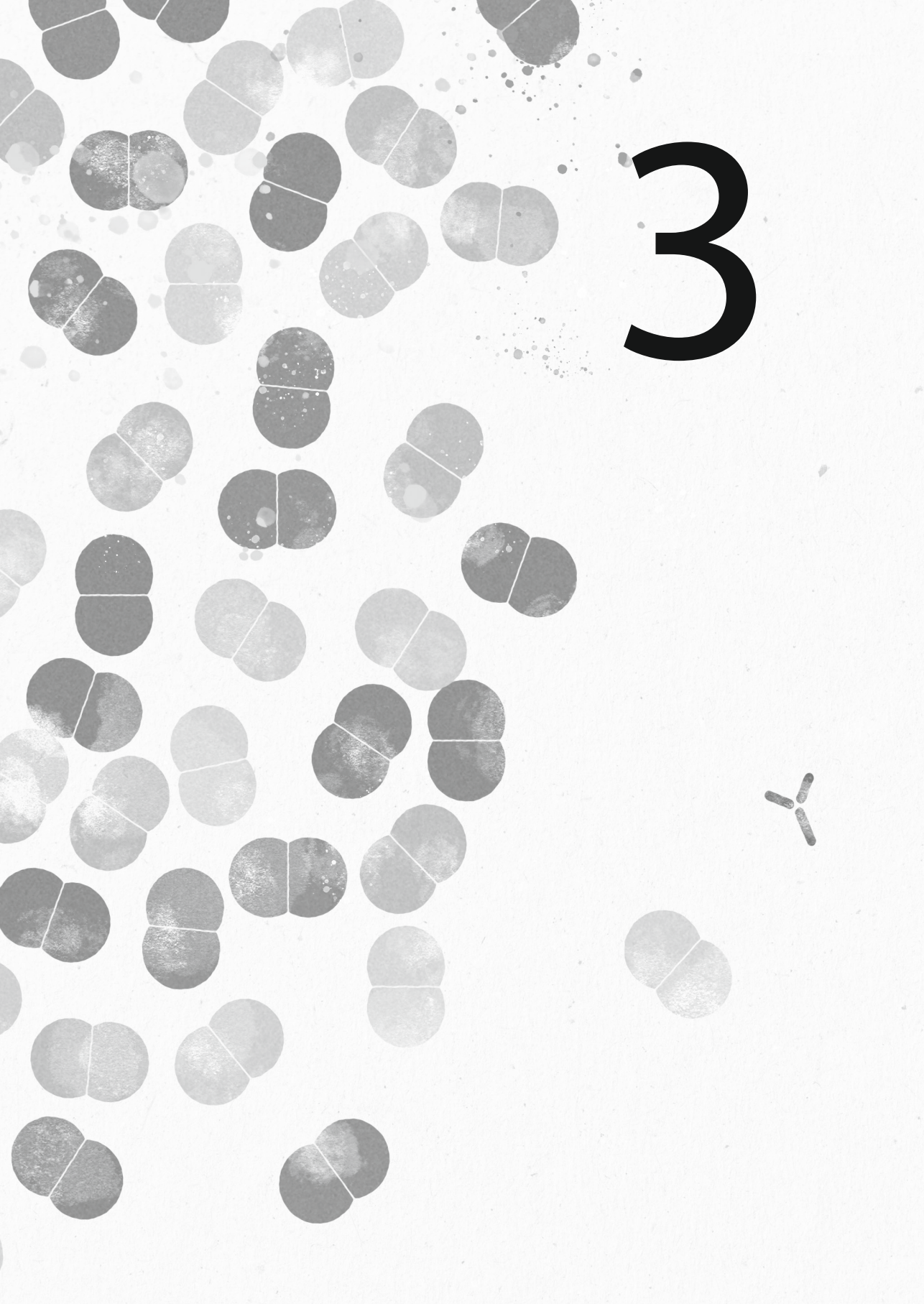

Vaccine impact and effectiveness of meningococcal serogroup ACWY conjugate vaccine implementation in the Netherlands: a nationwide surveillance study

Milou Ohm[1], Susan J M Hahné[1], Arie van der Ende[2], Elisabeth A M Sanders[1], Guy A M Berbers[1], Wilhelmina L M Ruijs[1], Nina M van Sorge[2], Hester E de Melker[1], Mirjam J Knol[1]

[1] Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands

[2] Department of Medical Microbiology and Infection Prevention and Netherlands Reference Laboratory for Bacterial Meningitis, Amsterdam UMC, location Amsterdam Medical Center, University of Amsterdam, Amsterdam, The Netherlands

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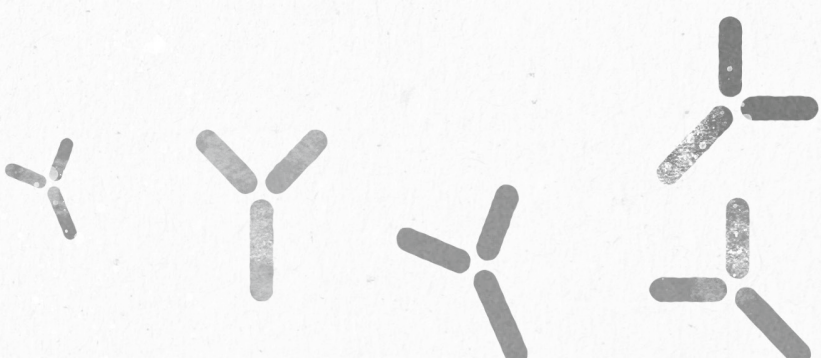

ABSTRACT

Background In response to the recent serogroup W invasive meningococcal disease (IMD-W) epidemic in the Netherlands, meningococcal serogroup C (MenC) conjugate vaccination for children aged 14 months was replaced with a MenACWY conjugate vaccination, and a mass campaign targeting individuals aged 14-18 years was executed. We investigated the impact of MenACWY vaccination implementation in 2018-2020 on incidence rates and estimated vaccine effectiveness (VE).

Methods We extracted IMD cases diagnosed between July 2014 and December 2020 from the national surveillance system. We calculated age group-specific incidence rate ratios by comparing incidence rates before (July 2017-March 2018) and after (July 2019-March 2020) MenACWY vaccination implementation. We estimated VE in vaccine-eligible cases using the screening method.

Results Overall, the IMD-W incidence rate declined by 61% (95% confidence interval [CI], 40 to 74). It declined by 82% (95% CI, 18 to 96) in the vaccine-eligible age group (individuals aged 15-36 months and 14-18 years) and by 57% (95% CI, 34 to 72) in vaccine-noneligible age groups. VE was 92% (95% CI, -20 to 99.5) in vaccine-eligible toddlers (aged 15-36 months). No IMD-W cases were reported in vaccine-eligible teenagers after the campaign.

Conclusions The MenACWY vaccination programme was effective in preventing IMD-W in the target population. The IMD-W incidence reduction in vaccine-noneligible age groups may be caused by indirect effects of the vaccination programme. However, disentangling natural fluctuation from vaccine effect was not possible. Our findings encourage the use of toddler and teenager MenACWY vaccination in national immunization programmes.

INTRODUCTION

Neisseria meningitidis, a gram-negative bacterium with a polysaccharide capsule that confers the specific serogroup, is an important cause of meningitis and septicaemia [1]. Worldwide, invasive meningococcal disease (IMD) is most often caused by serogroup A, B, C, W, X and Y [2]. The meningococcus can be carried asymptomatically in the nasopharynx, but can also act as harmful pathogen when crossing the mucosal barriers. Carriage rates are high in teenagers, which is attributed to factors like social behaviour including kissing and crowding [3, 4]. Although teenagers show low incidence rates in most infectious diseases [5], they are disproportionally affected by IMD, together with young children. On average, 1 in 10 patients dies from IMD in countries with excellent health care [6]. Furthermore, survivors may experience severe sequelae like deafness and limb amputation despite proper medical treatment [7]. Meningococci elude most of the host innate immune response and IMD can develop within hours. Hence, the host cannot rely on memory mechanisms that are important for a cellular response. Thus, circulating antibodies, together with the complement system, are essential for bacterial killing [8]. Vaccination is the best strategy to prevent disease by inducing such protective antibodies. The majority of currently applied meningococcal vaccines induce the production of antibodies that specifically target the meningococcal polysaccharide capsule.

A recent IMD serogroup W (IMD-W) epidemic in the Netherlands led to dozens of disease cases in individuals of all ages with a high mortality rate [9], caused by meningococci belonging to the hyperinvasive clonal complex 11 (cc11) [10]. This cc11 was already known for its ability to cause IMD-W epidemics in other countries such as the United Kingdom [11, 12]. To halt the epidemic, the meningococcal serogroup C conjugated to tetanus toxoid (MenC-TT) vaccine for toddlers was replaced by the MenACWY-TT vaccine in May 2018. In addition, a mass campaign in 2018-2019 that targeted individuals aged 14-18 years (birth cohort 2001-2005) was implemented, and the quadrivalent vaccine was introduced for all individuals aged 14 years in the national immunization programme (NIP) in 2020. This strategy aimed to directly protect these teenagers from disease and also limit transmission through this group [9].

Meningococcal vaccines are registered based on a serological correlate of protection that reflects the vaccine-induced immune response [13]. The reason is that rare diseases like IMD do not allow the use of clinical endpoints in pre-licensure studies that investigate vaccine efficacy directly. Consequently, post-licensure observational studies are necessary to evaluate effectiveness and impact of meningococcal vaccina-

tion [14]. Previous studies have proved that a mass campaign with a MenC conjugate vaccine targeting children can limit an epidemic [15]. However, comprehensive data on MenACWY vaccine effectiveness (VE) is lacking and it is unknown whether vaccinating only 14-18 year-olds restricts a national outbreak and induces herd immunity.

Here, we describe the impact of the MenACWY vaccination programme in the Netherlands between 2018 and 2020. We determined the impact of vaccination in different age groups by comparing nationwide incidence rates before and after the mass campaign, thereby investigating both direct and indirect protection. We report estimates of the VE in vaccine-eligible toddlers (aged 15-36 months) and teenagers (aged 14-18 years) in the Netherlands.

METHODS

IMD surveillance in the Netherlands

The national IMD surveillance system is based on 2 data sources: notifications from the Regional Public Health Service (RPHS) and laboratory data from the Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM, Amsterdam University Medical Center Amsterdam, the Netherlands). Data from these 2 sources are linked on a national level by the National Institute for Public Health and the Environment. In short, the notification of a case with clinical information from the RPHS, combined with a report of microbiological data including the serogroup from the NRLBM, results in a complete overview of all nationally occurring IMD cases. Linking between the 2 sources was possible for 87% of all unique records, as described previously [16].

A case was defined as a positive sample from a sterile site confirmed by culture, by polymerase chain reaction, or both. Vaccination status of each case was obtained from the national vaccination registry. Cases were only included in mortality analyses if the outcome status was known. A vaccine failure was defined according to the World Health Organization guidelines as follows: a laboratory-confirmed meningococcal case with onset more than 10 days after the scheduled dose of the vaccine targeting the respective disease-causing serogroup [17]. The national electronic vaccination register monitors the vaccination status for all minors up to age 18 years. The routine coverage in children aged 14 months was estimated at 93%, based on yearly published vaccine coverage data from this register [18]. The vaccine coverage within the teenager mass campaign was previously estimated at 86% [19].

Periods for impact analyses

Epidemiological years were used to describe IMD cases from 2014 to 2020, with a year starting July 1 and ending June 30 the year thereafter. The period of quartile 3 (Q3)-2017 until Q1-2018 was chosen as period before implementation because of corresponding length and seasonal characteristics as the period after implementation (Figure 1). The period also reflects the epidemiology of disease during the epidemic well. By only including the period during the peak of the IMD-W epidemic, the risk of underestimating the impact was limited. The period after implementation was defined as starting Q3-2019 and ended at Q1-2020 to limit interference of the measures taken, starting close to Q2-2020, to control the coronavirus disease 2019 (COVID-19) pandemic. Data from Q2-2020 until Q4-2020 were also analyzed to determine the effect of the COVID-19 containment measures on IMD incidence. A sensitivity analysis repeated the before-after analyses but additionally included Q2-18 and Q3-2018 in the period before implementation, in order to evaluate to what extent the chosen period affected the estimated impact (Figure 1). This sensitivity period included the period when the MenACWY-TT vaccine was already implemented for children aged 14 months, but the mass campaign for teenagers had not yet started.

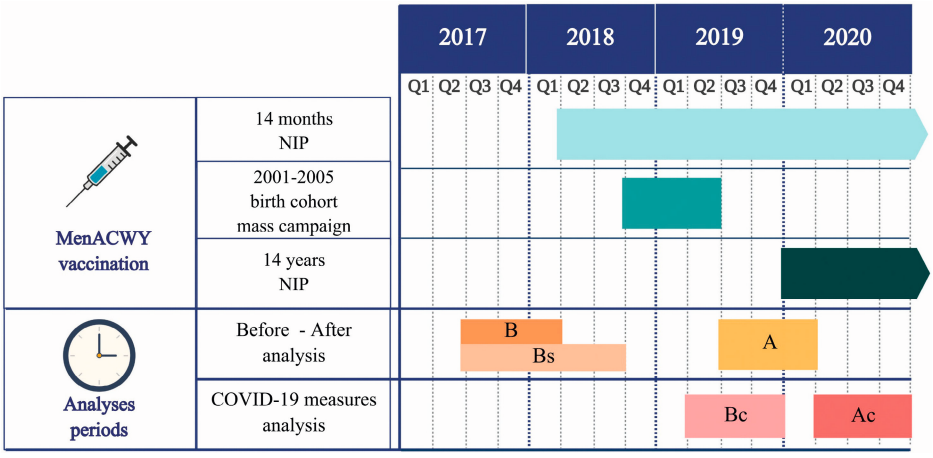

Figure 1. Timeline of implementation and analyzed periods. Abbreviations: A, post-implementation period; Ac, period with COVID-19 containment measures; B, pre-implementation period, base case analyses; Bc, period before COVID-19 containment measures; Bs, pre-implementation period within the sensitivity analysis; COVID-19, coronavirus disease 2019; MenACWY, meningococcal serogroup A, C, W and Y; NIP, national immunization program; Q, quartile. Created with BioRender.com.

Statistical analysis

The impact of the MenACWY vaccination campaign was analyzed by comparing incidence rates per 100 000 individuals per year in periods before and after implementation (Figure 1), expressed as incidence rate ratio (IRR). We estimated the impact for different serogroups within different age groups and for the whole population. We calculated 95% confidence intervals (CIs) of IRR using a Poisson regression model. Age groups were categorized in accordance with the vaccination programme, individuals aged 15-36 months and 14-18 years were defined as the vaccine-eligible age groups, and individuals aged <15 months, 3-13 years and >19 years were defined as the vaccine-noneligible age groups. Since IMD-B is not targeted by the vaccine, this serogroup was included in the impact analysis as means of a negative control.

The VE was assessed for laboratory-confirmed IMD-W cases in vaccine-eligible toddlers and teenagers. Vaccine eligibility in toddlers was defined as born on or after 1 March 2017 and diagnosed at age ≥14 months between 1 May 2018 and 31 December 2020. Vaccine-eligible teenagers were born between 1 January 2001 and 31 December 2005 and diagnosed between 1 July 2019 and 31 December 2020 at age ≥14 years. We calculated the VE by comparing the proportion of cases vaccinated to the proportion of the population vaccinated in the studied cohort, which is the vaccine coverage in the respective cohort, using the screening method [20] with the following formula:

$$VE = 1 - \frac{PCV}{1 - PCV} * \frac{1 - PPV}{PPV}$$

where *PCV* is the proportion of cases vaccinated in the studied cohort and *PPV* is the proportion of the population vaccinated.

Data on population size were obtained from Statistics Netherlands to calculate incidence per population time. Population data for 2020 was not yet available at time of analyses (January 2021); therefore, population data from 2019 were used to calculate population size for 2020. Statistical analyses were performed using Excel, GraphPad Prism 8 and SPSS Statistics 24.

RESULTS

A total of 884 IMD cases was reported in a 6-year period from 2014-15 until 2019-20 (Figure 2). IMD cases were predominantly caused by serogroup B in 2014-15 (Figure 2) and the years before (data not shown). While only 5 cases of IMD-W were observed in 2014-15, it was the most common serogroup in 2017-18 with 104 cases. IMD-C has rarely been observed since the introduction of MenC vaccination in 2002, with only a few cases occurring throughout the studied years (Figure 2). IMD-Y accounted for 12% (n=109) of all cases in the period 2014-15 to 2019-20, whereas IMD due to other serogroups such as IMD-E and IMD-X, and non-groupable IMD accounted for a few cases per year (data not shown). In the studied period, IMD-A was never reported. The largest proportion of fatal IMD-W cases in the study period occurred in 2017-18 (47%; 22 out of 47 cases). Of 22 deceased cases in 2017-18, 13 (59%) were adults aged ≥45 years and 6 were individuals aged between 14 and 24 years. In 2019-20, only 3 fatal IMD-W cases were reported.

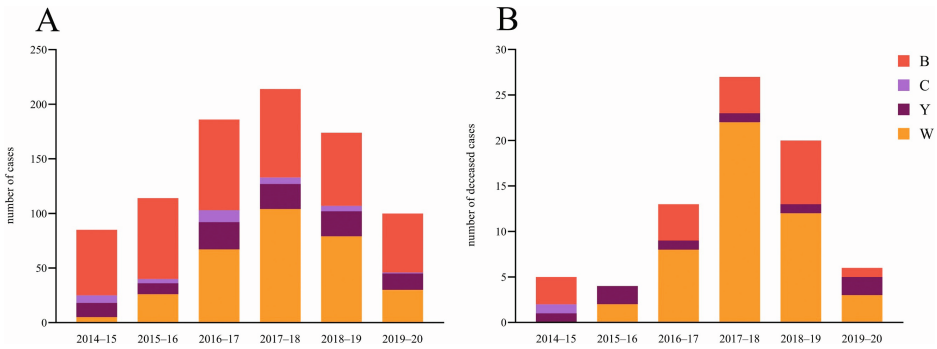

Figure 2. Number of invasive meningococcal serogroup B, C, Y, and W disease cases (A) and deceased cases* (B) in the period 2014–2015 to 2019–2020. *Only cases with known outcome status are shown (outcome status missing for 12 invasive meningococcal disease [IMD]-B cases, 0 IMD-C cases, 8 IMD-Y cases, and 7 IMD-W cases in this 6-year period).

While IMD-W cases were rare and only observed in adults in 2014-15, incidence started to increase in 2015-16 with highest incidence in children ≤15 months of age, albeit low absolute numbers (Figure 3). In 2016-17, incidence increased, particularly in individuals aged 14-18 years (0.20 to 1.07 per 100 000) followed by a rise in incidence in almost all age groups in the year thereafter. Children aged ≤36 months were disproportionally affected during the peak years, although the absolute number of cases was highest in middle-aged adults and elderly (Figure 3).

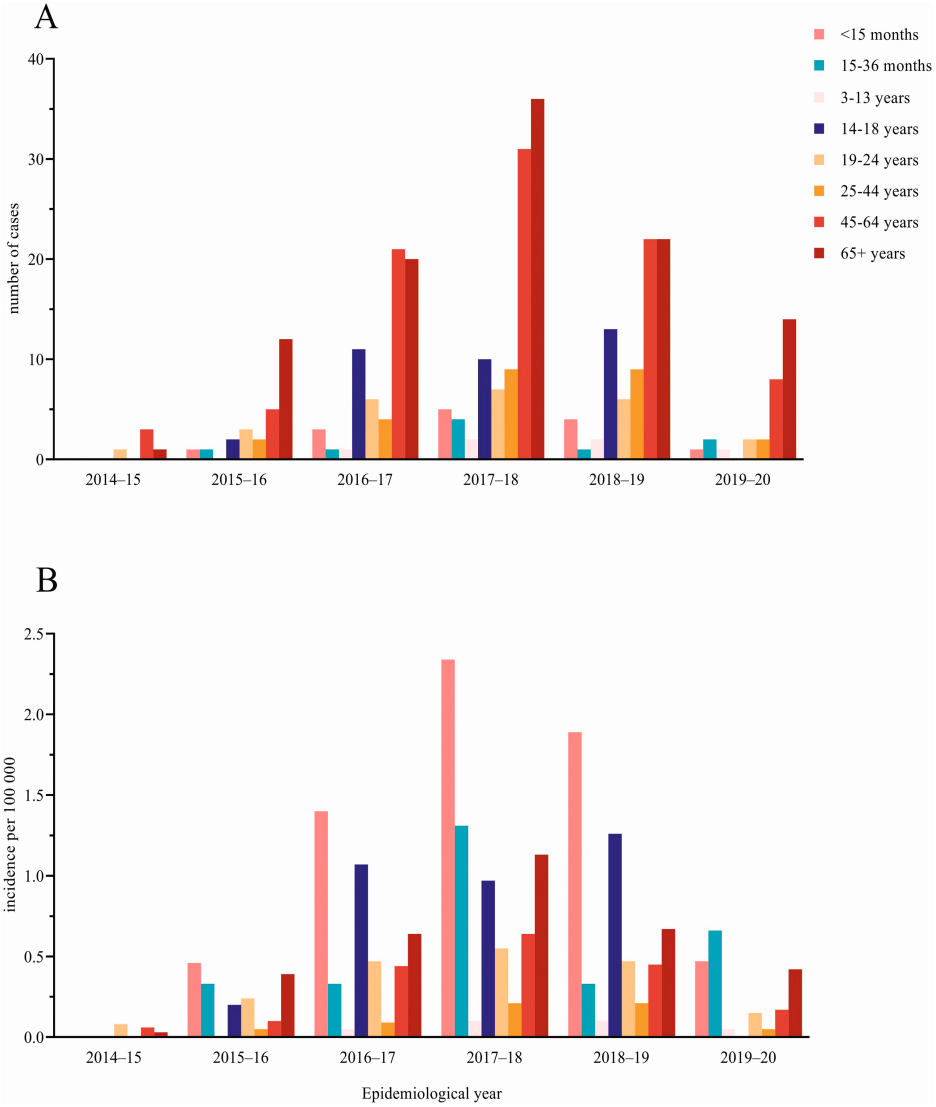

Figure 3. Number of cases (A) and incidence (B) of invasive meningococcal disease W per age group.

The number of cases dropped in 2018-19 in all age groups except in individuals aged 14-18 years, with 13 cases that year compared with 10 cases the year before. Over the years, the number of cases and incidence rates were continuously low in individuals aged 3-13 years and 25-44 years.

During the mass campaign, the incidence in vaccine-eligible groups rapidly declined (Figure 4). After the mass campaign, the IMD-W incidence rate had declined in all age groups (Table 1). The most pronounced reduction was observed in vaccine-eligible individuals aged 14-18 years with 8 cases before implementation and zero after implementation. Older age cohorts (adults aged 45-64 years and ≥65 years) also showed a significant decrease in incidence; the overall IRR for the vaccine non-eligible age groups was 0.43 (0.28-0.66).

Table 1. Incidence rate (IR) and incidence rate ratio (IRR) for meningococcal serogroup W per age group and for serogroup B, C and Y per vaccine-eligible or vaccine-noneligible age group, in the period before and after implementation of meningococcal A, C, W and Y conjugated to tetanus toxoid (MenACWY-TT) vaccination.

	Age group	N	IR Q3-2017 to Q1-2018 (before)	N	IR Q3-2019 to Q1-2020 (after)	IRR	95% CI
Serogroup W	<15 months	4	2.49	1	0.63	0.25	0.03–2.27
	15-36 months	3	1.31	2	0.88	0.67	0.11–4.02
	3-13 years	0	0.0	1	0.07	NA	NA
	14-18 years	8	1.03	0	0.0	NA	NA
	19-24 years	5	0.52	2	0.21	0.39	0.08–2.03
	25-44 years	7	0.22	3	0.09	0.42	0.11–1.64
	45-64 years	24	0.66	8	0.22	0.33	0.15–0.74
	≥65 years	27	1.13	14	0.56	0.50	0.26–0.95
	All	78	0.61	31	0.24	0.39	0.26–0.60
	Vaccine-eligible	11	1.09	2	0.20	0.18	0.04–0.82
	Vaccine-noneligible	67	0.57	29	0.24	0.43	0.28–0.66
Serogroup C	Vaccine-eligible	0	0.0	0	0.0	NA	NA
	Vaccine-noneligible	5	0.04	1	0.01	0.20	0.02–1.69
Serogroup Y	Vaccine-eligible	2	0.20	0	0.0	NA	NA
	Vaccine-noneligible	15	0.13	14	0.12	0.92	0.45–1.91
Serogroup B	Vaccine-eligible	18	1.79	19	1.90	1.06	0.56–2.02
	Vaccine-noneligible	46	0.39	30	0.25	0.65	0.41–1.02

Vaccine-eligible, aged 15–36 months and 14–18 years; vaccine-noneligible, aged under <15 months, 3–13 years, and ≥19 years. Abbreviations: IR, incidence rate; IRR, incidence rate ratio; NA, not applicable; Q, quartile.

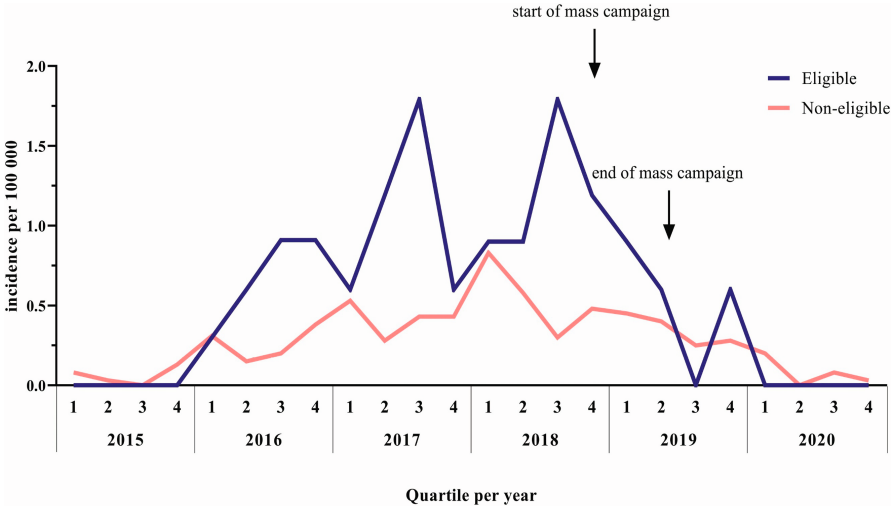

Figure 4. Invasive meningococcal disease W incidence per quartile during the calendar years 2015–2020 in vaccine-eligible (aged 15–36 months and 14–18 years) and vaccine-noneligible (aged <15 months, 3–13 years, and ≥19 years) groups.

After implementation of the MenACWY vaccination, IMD-Y cases were absent in age groups eligible for vaccination; this is in contrast to 2 cases in the period before implementation (Table 1). In vaccine-noneligible age groups, no difference was observed in IMD-Y incidence (IRR 0.92). Although IMD-C cases were already rare and only observed in individuals aged ≥45 years, there were even fewer cases after implementation of MenACWY vaccination (5 before, 1 after). Overall, the impact on total MenACWY cases was larger in vaccine-eligible age groups than in vaccine-noneligible age groups (IRR, 0.15; 0.03-0.68 and IRR, 0.50; 0.35-0.72, respectively) though all age groups showed a decreasing incidence (data not shown). The incidence of IMD-B did not change in vaccine-eligible age groups and decreased slightly but not significantly in vaccine-noneligible age groups.

A sensitivity analysis was carried out by including 2 additional quartiles (Q2-2018 and Q3-2018) in the period before implementation and showed that IRRs did not change when the analyzed period included this extended period before implementation (Supplementary Table 1). The incidence of IMD-W during COVID-19 containment measures (Q3-Q4 2020) was lower than in Q3-Q4 2019 (Figure 4), a period just after implementation of the vaccination with the same seasonal characteristics but before COVID-19 measures were taken (Figure 1). The incidence of IMD-B also decreased during the time COVID-19 measures were in place, although the decrease in noneligible age groups was less pronounced than for IMD-W and IMD-Y (Table 2).

Table 2. Incidence rate (IR) and incidence rate ratio (IRR) for meningococcal serogroup W, Y and B per vaccine cohort (vaccine-eligible, vaccine-noneligible and overall), comparing period before and during COVID-19 containment measures.

	Cohort	N	IR Q3-19 to Q4-19 (before COVID)	N	IR Q3-20 to Q4-20 (during COVID)	IRR	95% CI
Serogroup W	Vaccine-eligible	2	0.30	0	0.0	NA	NA
	Vaccine-noneligible	21	0.26	4	0.05	0.19	0.07–0.55
	Overall	23	0.27	4	0.05	0.17	0.06–0.50
Serogroup Y	Vaccine-eligible	0	0.0	0	0.0	NA	NA
	Vaccine-noneligible	6	0.08	1	0.01	0.17	0.02–1.38
	Overall	6	0.07	1	0.01	0.17	0.02–1.38
Serogroup B	Vaccine-eligible	13	1.95	3	0.45	0.19	0.07–0.55
	Vaccine-noneligible	18	0.23	14	0.18	0.67	0.37–1.21
	Overall	31	0.36	17	0.20	0.55	0.30–0.99

Vaccine-eligible, aged 15–36 months and 14–18 years; vaccine-noneligible, aged under <15 months, 3–13 years, and ≥19 years. Abbreviations: IR, incidence rate; IRR, incidence rate ratio; NA, not applicable; Q, quartile.

The estimated VE for 1 dose of MenACWY-TT in children aged 14 months against IMD-W was 92% (95% CI, -20 to 99.5). Two IMD-W cases occurred in this eligible cohort, both aged ≥14 months at the time of diagnosis and eligible for vaccination based on date of birth (being born after March 2017). One case was vaccinated 16 months prior to becoming ill, and 1 was unvaccinated. No IMD-W cases were observed in teenagers eligible for vaccination; therefore, VE could not be estimated in this cohort. For the other serogroups included in the vaccine (serogroup ACY), it was also not possible to estimate the VE due to the lack of cases in both vaccine-eligible cohorts.

DISCUSSION

In response to a national IMD-W epidemic in the Netherlands, MenACWY vaccination was implemented in the NIP for toddlers from April 2018 onward and for teenagers from October 2018 onward, together with a mass campaign for individuals aged 14-18 years between October 2018 and June 2019. In this study, we evaluated IMD cases in the Netherlands from 2014-15 onward, at the time the IMD-W epidemic emerged and the NIP consequently was adjusted to counter the epidemic. We found an overall 61% decrease in IMD-W incidence and an even higher reduction of cases of 82% in vaccine-eligible toddlers and teenagers, within the first year after the mass campaign was completed. The VE in toddlers was 92%; only 1 vaccinated toddler became ill with IMD-W. No cases were observed in teenagers after the mass campaign, thereby pre-

cluding an estimate of VE in this cohort. Whereas incidence of the vaccine-preventable serogroup Y did decrease in the vaccine-eligible cohort, there was very little decline in IMD-Y in vaccine-noneligible age groups (IRR, 0.92) in the first 3 quartiles after completion of the mass campaign.

A catch-up programme in the UK between 2015 and 2017 provided the MenACWY vaccination to all individuals aged 13-18 years [21]. Despite a low coverage of 36.6% in the first cohort to be vaccinated, 69% fewer IMD-W cases were observed than were predicted to occur without intervention during the first 12 months of the teenager MenACWY vaccination programme [22]. Comparable to our findings in toddlers, the early estimated VE in teenagers in that study was 100% for IMD-W, but with wide confidence intervals (9%CI -47 to 100) due to small numbers. A study from Chile showed a 92% reduction in IMD-W cases in the first 4 years after a mass campaign in the MenACWY vaccinated cohort that consisted of infants and children aged 9 months to 4 years [23]. Indirect effects were not yet observed 1 year after vaccination in Chile; the lack of infants younger than 9 month of age and teenagers in the target group was given by the authors as possible explanation. Several European countries reported an increase in IMD-W during the years 2013-17; however, the Netherlands was among the most strongly affected countries [12], and one of the few that implemented the MenACWY vaccination in response to the epidemic. In less affected countries, implementation was considered but often not recommended by National Immunization Technical Advisory Groups for benefit, risk, and cost reasons.

Most studies that investigated the effectiveness of the monovalent MenC conjugate vaccine reported VE results that were similar to what we observed for the quadrivalent MenACWY conjugate vaccine. According to a systematic review that studied meningococcal transmission and disease in adolescents, MenC-TT effectiveness was approximately 90% within the first year post-vaccination [24]. The effectiveness of MenC-TT in the routinely vaccinated cohort in England (3 doses given to infants aged 2-4 months) was 93% within 1 year of the scheduled vaccination [25]. In Italy since 2005, a major reduction of cases has been observed after a single dose of MenC-TT was provided at age 13-15 months, with some regions carrying out mass-campaigns with either MenC or MenACWY conjugate vaccinations in the years thereafter [26]. Overall, high VE of the MenC-TT vaccine has been observed in the past across different European countries, with vaccine failure begin rare.

Since the start of the COVID-19 containment measures in March 2020, partial lockdowns did not only reduce COVID-19 disease, they also reduced the incidence of many

other infectious diseases [27, 28]. At the time of COVID-19 containment measures, which was more than 1 year after the MenACWY mass campaign was completed, all serogroup IMD incidence decreased substantially. As a consequence of those measures, we could only include a constrained period in our before-after analysis, with both periods consisting of 3 quartiles. The analysis showed a decrease in IMD-W incidence in vaccine-noneligible age groups, suggesting a herd effect. However, stabilization of the incidence had already appeared at the start of the mass campaign. In addition, we did not find any early impact in vaccine-noneligible groups for other vaccine-targeted serogroups such as IMD-Y, but the number of cases was low. Remarkably, in vaccine-noneligible age groups, the decrease in IMD-W and IMD-Y incidence (IRR, 0.19; 0.07-0.55 and IRR, 0.17; 0.02-1.38, respectively) during the period with COVID-19 measures was larger than for IMD-B (IRR, 0.67; 0.37-1.21), which is not covered by the vaccine. This could be supportive for an additional effect of group immunity by MenACWY vaccination. However, the epidemiology of IMD-B is different from IMD-W and IMD-Y, for example, in terms of age-related susceptibility, and the decrease in IMD-B in vaccine eligible groups was similar to IMD-W and IMD-Y in vaccine-noneligible age groups during the measures with IRR, 0.19 (0.07-0.55). Thus, the significance of these findings remains uncertain.

One drawback of observational research is that it may be confounded by natural trends in the incidence of disease over time. Meningococci are known for seasonal variation [29], and incidence varies not only within a year, but also throughout the years. For example, IMD-B incidence has been steadily declining since early 2000 in the Netherlands without demonstrable reason; in contrast IMD-W suddenly increased rapidly in 2015-16. This highlights the importance of comparing periods with the same seasonality, if available, and a critical appraisal of the periods chosen for the before-after analysis. Our sensitivity analysis showed that the period chosen for analysis, although it consisted of only 3 quartiles, was robust for the impact analyses. However, as possible explanation for the observed decrease, we cannot rule out that natural changes in epidemiology may have added to a vaccine-induced effect. Carriage studies should verify if the vaccination campaign truly led to the proposed herd effect through reduced transmission, although behavioral factors such as intimacy with others and smoking may also affect carriage rates [4]. Evidence for reduced meningococcal carriage after a quadrivalent vaccine is present but limited [30] and sometimes controversial. A cross-sectional carriage study in the United Kingdom in university students observed a substantial rise in meningococcal serogroup W carriage despite a coverage of 71% with the MenACWY-TT vaccine [31]. It should, however, be taken into account that this study investigated a close-contact and thus high-risk setting. Also, a recent modelling

study using the same carriage data showed that vaccination led to a carriage plateau, and the authors predicted that a higher coverage rate would have produced further reduction in carriage levels [32].

In conclusion, we found that the implementation of a MenACWY conjugate vaccine for individuals aged 14-18 years through a mass campaign, in addition to its introduction in the NIP for toddlers and teenagers, led to a reduction in IMD-W cases in vaccine-eligible age groups. A decline in IMD-W incidence was also observed in vaccine-noneligible groups, but it remains uncertain to what extent the reduction can be attributed to indirect effects of the vaccination campaign because it is difficult to disentangle natural fluctuation from vaccine effect. This study provides information for countries facing an IMD-W epidemic and highlights the importance of continuous surveillance to improve vaccination policies and enable quick intervention during an outbreak. It underlines the high effectiveness of MenACWY vaccination and encourages its use as toddler and teenager vaccination in national immunization programmes.

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Supplementary Table 1. Incidence rate (IR) and incidence rate ratio (IRR) comparing the sensitivity period before and after implementation of meningococcal A, C, W and Y conjugated to tetanus toxoid (MenACWY-TT) vaccination for meningococcal serogroup W per age group, and per vaccine-eligible or vaccine-noneligible age group.

	Age group	N	IR Q3-2017 to Q3-2018 (before)	N	IR Q3-2019 to Q1-2020 (after)	IRR	95% CI
Serogroup W	<15 months	6	2.24	1	0.63	0.28	0.03–2.34
	15-36 months	5	1.32	2	0.88	0.67	0.13–3.45
	3-13 years	4	0.16	1	0.07	0.42	0.05–3.77
	14-18 years	15	1.16	0	0.0	NA	NA
	19-24 years	9	0.56	2	0.21	0.36	0.08–1.69
	25-44 years	10	0.19	3	0.09	0.50	0.14–1.80
	45-64 years	36	0.60	8	0.22	0.37	0.17–0.80
	≥65 years	37	0.92	14	0.56	0.61	0.33–1.13
	All	122	0.57	31	0.24	0.42	0.28–0.62
	Vaccine-eligible	20	1.19	2	0.20	0.17	0.04–0.72
	Vaccine-noneligible	102	0.52	29	0.24	0.47	0.31–0.71

Vaccine-eligible, aged 15–36 months and 14–18 years; vaccine-noneligible, aged under <15 months, 3–13 years, and ≥19 years. Abbreviations: CI, confidence interval; NA, not applicable; Q, quartile.

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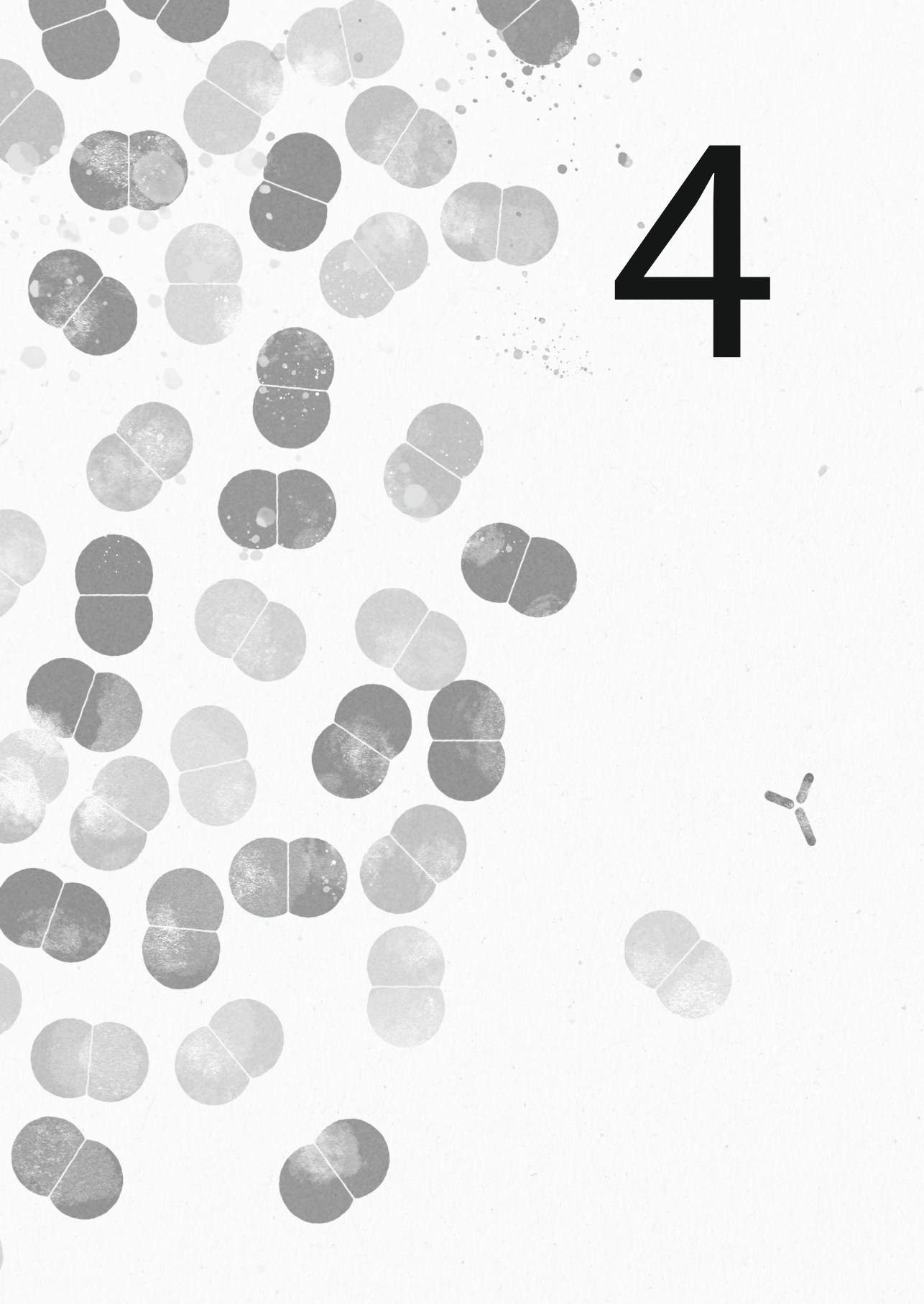

Different long-term duration of seroprotection against *Neisseria meningitidis* in adolescents and middle-aged adults after a single meningococcal ACWY conjugate vaccination in the Netherlands

Milou Ohm[1], Debbie M. van Rooijen[1], Axel A. Bonačić Marinović[1], Mariëtte B. van Ravenhorst[2], Marieke van der Heiden[3], Anne-Marie Buisman[1], Elisabeth A M Sanders[1], Guy A M Berbers[1]

[1] Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands

[2] Department of Pediatrics, Amsterdam UMC, Amsterdam, The Netherlands

[3] Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Stockholm, Sweden

[4] Department of Pediatric Immunology and Infectious Diseases, Wilhelmina Children's Hospital, University Medical Center, Utrecht, the Netherlands

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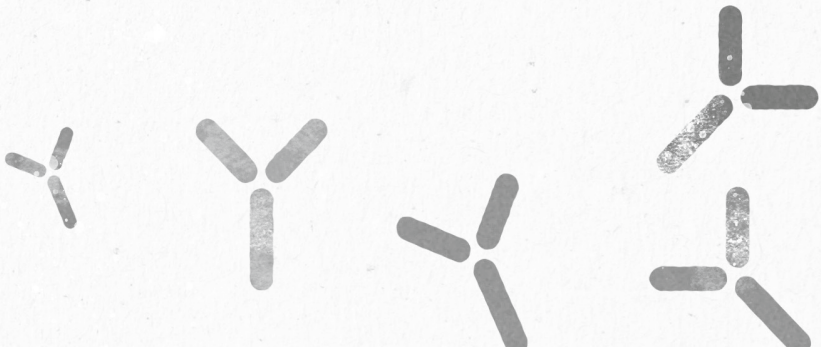

ABSTRACT

Neisseria meningitidis is often asymptomatically carried in the nasopharynx but may cause invasive meningococcal disease, leading to morbidity and mortality. Meningococcal conjugate vaccinations induce functional protective antibodies against capsular antigens, but seroprotection wanes over time. We measured functional antibody titers five years after administration of a single dose of the meningococcal ACWY-polysaccharide-specific tetanus toxoid-conjugated (MenACWY-TT) vaccine in adolescents and middle-aged adults in the Netherlands, using the serum bactericidal antibody with baby rabbit complement (rSBA) assay. Protection was defined as rSBA titer ≥8. The meningococcal ACWY-specific serum IgG concentrations were measured with a multiplex immunoassay. Duration of protection was estimated by a bi-exponential decay model. Sufficient protection for MenC, MenW, and MenY was achieved in 94–96% of the adolescents five years postvaccination, but, in middle-aged adults, only in 32% for MenC, 65% for MenW and 71% for MenY. Median duration of protection for MenCWY was 4, 14, and 21 years, respectively, in middle-aged adults, while, in adolescents, it was 32, 98, and 33 years. Our findings suggest that adolescents, primed in early childhood with MenC conjugate vaccination, remain sufficiently protected after a single dose of MenACWY-TT vaccine. Middle-aged adults without priming vaccination show fast waning of antibodies, particularly MenC, for which protection is lost after four years.

INTRODUCTION

As a commensal bacterium, *Neisseria meningitidis* resides in the nasopharynx in humans mostly without clinical symptoms. However, sometimes encapsulated serogroups may invade the bloodstream of the human host, and cause invasive meningococcal disease (IMD) [1, 2]. IMD has both severe acute and life-long consequences and is a major cause of mortality [3]. Antibiotics are the main therapy, together with supportive therapy, but may work too late against this rapidly progressive disease. To prevent meningococcal disease, vaccination with meningococcal conjugate vaccines induces the production of protective antibodies against the polysaccharide capsule of the meningococcal bacterium [4]. Although a vaccine also induces a cellular memory response, the response might be too slow to provide protection against *Neisseria meningitidis.* A memory response can take up to five days, while an invasive disease can manifest itself within hours after encountering the pathogen [5, 6]. For protection, it is therefore necessary to maintain sufficient levels of circulating anticapsular antibodies that directly interact with the complement system to prevent invasive disease by bacterial killing [7-10].

A steep rise in meningococcal C (MenC) disease incidence around 2000 in the Netherlands led to the introduction of a single MenC tetanus toxoid conjugate (MenC-TT) vaccination at 14 months of age in the national immunization program (NIP) in 2002 [11]. In addition, a MenC-TT vaccination was offered to all children aged 1–18 years as part of a catch-up mass-campaign to eradicate MenC circulation. A single-dose schedule at 14 months of age did not provide sufficient protection on the long-term [12] and the timing of an adolescent booster-dose in the NIP was investigated [13, 14]. In 2015/2016, a MenW epidemic in the Netherlands emerged, which led to the introduction of meningococcal A, C, W, and Y conjugated to tetanus toxoid (MenACWY-TT) vaccine for toddlers at age 14 months with a booster vaccination at the age of 14 years. At the same time, a catch-up campaign with a single dose of the MenACWY-TT vaccine was conducted in all 14 to 18-year-olds. Although this campaign is assumed to include the main carriers of MenW [15] and, in this way, might eventually lead to a benefit for unvaccinated individuals through herd protection; this may take time [16]. Disease cases in the MenW epidemic occurred not only in the very young children and in adolescents, but also in adults and in the elderly [17]. To protect older age groups directly against IMD, vaccination of other age categories might be required [18]. In former studies, MenACWY-TT vaccination has shown to elicit a good immune response in both children and adults [18-24]. However, we have previously shown that the functional antibody titers in middle-aged adults were lower compared to adolescents already at one month after vaccination [25].

To optimize vaccination strategies, knowledge about the duration of protection after a single MenACWY-TT vaccination is essential. However, long-term persistence of functional antibodies induced by a MenACWY-TT vaccination has been scarcely investigated, especially in older adults [26, 27]. The level of vaccine-induced protection and potentially also the duration of protection seems to vary among different age groups [22, 28]. The aim of the current study was to determine duration of protection after a single MenACWY-TT vaccination in adolescents who were once primed with a single MenC-TT vaccination at preschool age and middle-aged adults who were naïve to meningococcal vaccination. We assessed both functional antibody titers and concentrations of IgG antibodies using a five-year postvaccination serum sample and estimated vaccine-induced duration of protection in years using a multilevel bi-exponential decay model. Furthermore, meningococcal type-specific IgG concentrations were compared with rSBA titers of the corresponding serogroup to gain insight into the difference between quantity and functionality of persisting antibodies.

METHODS

Study design and participants

This is a five-year follow-up study of two phase-IV trials conducted in a single center in the Netherlands. In these trials, a primary MenACWY-TT vaccination was administered to 225 healthy adolescents who were all once primed with a MenC-TT vaccine (NeisVac-C) at an age between 14 months and 3 years, and to 204 healthy middle-aged adults who were naïve to meningococcal vaccination. Detailed information on recruitment, study design, in-and exclusion criteria and clinical procedures are previously described [18, 29]. In short, in the adolescent trial (EudraCT number: 2013-001823-38, Dutch Trial Register: NL4286), healthy 10-, 12-, and 15-year-olds were recruited in the surrounding area of Utrecht, the Netherlands. All participants received a single dose of the MenACWY-TT vaccine in the spring of 2014. Venous blood samples were collected before, 1 month, and 1 year after the study vaccination. In the middle-aged adult trial (EudraCT number: 2014-000967-42, Dutch Trial Register: NL4518), healthy 50- to 65-year-olds were recruited in the municipality of Amersfoort, the Netherlands. All participants received a single dose of the MenACWY-TT vaccine in the autumn of 2014. Venous blood samples were collected before, 7 days, 1 month, and 1 year after the study vaccination.

In the follow-up studies, all participants that completed the former trial and gave permission to approach them in the future were asked to donate a venous blood sample that was collected 5 years (±3 months) after vaccination. Receiving an additional MenACWY vaccination after the 1-year timepoint was now added to the exclusion criteria. Participants that failed to build up an immune response after the MenACWY-TT vaccination during the adolescent study were excluded because they were offered an extra vaccination.

These studies were designed and conducted in accordance with the Good Clinical Practice guidelines established by the International Conference on Harmonization and with the Declaration of Helsinki. Ethical approval was obtained from the local ethics committee Medical research Ethics Committees United (MEC-U) for both follow-up studies. The middle-aged adult study was approved as an amendment. Since the adolescent study was already officially terminated in the national study register, this follow-up study was registered separately at the Dutch Trial Register (NL7735). Written informed consent was obtained from all participants and from both parents or guardians when a subject was aged <16 years at enrolment.

Serological analysis

The functional antibodies were assessed by performing a serum bactericidal antibody with baby rabbit complement (rSBA) assay (Pelfreez, Rogers, Arkansas, U.S.A, lot 22841) and MenC strain C11 [30], MenW strain MP01240070, and MenY strain S-1975 as target strains. The serum bactericidal titer was defined as the dilution of the test serum that yielded ≥50% killing after 60 min incubation with a titer of ≥8 as correlate of protection [31-33]. Functional antibody titers were also analyzed using the more conservative threshold of ≥128 [31, 32]. For statistical purposes, rSBA titers below the cut-off of the assay (<4) were given a value of 2. Since no data were available from the former study in middle-aged adults about MenA titers, the MenA rSBA assay was not performed in this follow-up study. MenA-, MenC-, MenW-, and MenY-PS specific serum IgG concentrations were measured using the fluorescent-bead-based multiplex immunoassay (MIA) as previously described [34-36], with minor modification of using a protein-free buffer (Surmodics, Eden Prairie, MN, U.S.A.) since 2019. The lower limit of quantitation was assigned at 0.01 µg/mL for all four serogroups [34-36]. A previously suggested, arbitrary cut-off of ≥2 µg/mL for total serum IgG was used for analyses [37-41].

Mathematical model

A multilevel bi-exponential decay model was used to estimate the long-term protection in terms of functional antibody persistence [42-45]. This model describes the rSBA titer decay with the following equation:

$$Y(t) = Y_1\left(\frac{e^{-\nu_1(t-t_1)} + f_y e^{-\nu_2(t-t_1)}}{1+f_y}\right)$$

where $Y(t)$ is the antibody titer as function of time (t) after reaching its peak concentration at time t_1. ν_1 and ν_2 are the rates of the two exponential decay components conforming the bi-exponential model. After the antibody level peaks at time t_1 with value Y_1, its decay rate is dominated by the faster decay rate ν_1 . After a while, depending on the value of the factor f_y, the antibody level decay rate slows down and ends up being dominated by the slower decay rate, ν_2. The individual antibody titers of each participant at four timepoints (just before, one month, one year, and five years after vaccination) were used to inform the model under a Bayesian framework. By means of Markov chain Montecarlo simulations, the model parameters were calculated and used to predict expected rSBA titers as a function of time. Four million iterations per simulation were calculated using the software JAGS [46], version 4.3, run under R, version 3.6.3 (https://www.r-project.org/).

Statistical analysis

All statistical analyses were performed using GraphPad Prism 8 and SPSS Statistics 24. rSBA geometric mean titers (GMTs) and geometric mean concentrations (GMCs) of the meningococcal specific IgG concentrations were calculated with corresponding 95% confidence intervals (CI). Differences between age groups in GMTs and in GMCs at the five-year timepoint, and for the GMTs also at the pre-vaccination timepoint (T0) were determined with the Mann–Whitney test. Differences between age groups in GMTs at the timepoints one month and one year postvaccination were determined with linear regression analyses on natural log-transformed values, adjusting for pre-vaccination values from the former studies. Proportions with 95% CI of participants with a rSBA ≥8 and ≥128 were calculated using the Wilson score interval with continuity correction [47]. Differences in proportions at the five-year timepoint were tested with the Fisher's exact test. The Spearman correlation coefficient (R) was calculated to compare rSBA titers and IgG concentrations. Statistical tests were 2-sided. A calculated *p*-value below 0.05 was considered statistically significant.

RESULTS

Study participants

Of the 225 participants that received a MenACWY-TT vaccination in the adolescent study [29], 221 were approached with an invitation to participate again. Many former participants who were interested in participating had to be excluded from this study due to a recently received MenACWY-TT vaccination as part of the mass-campaign in the Netherlands; therefore, only 50 could be included in the current follow-up study. Of the 204 participants in the middle-aged adult study [18], 194 were approached with an invitation to participate again and 130 could be included in the follow-up study. In total, 180 participants were enrolled in the follow-up studies (Figure 1).

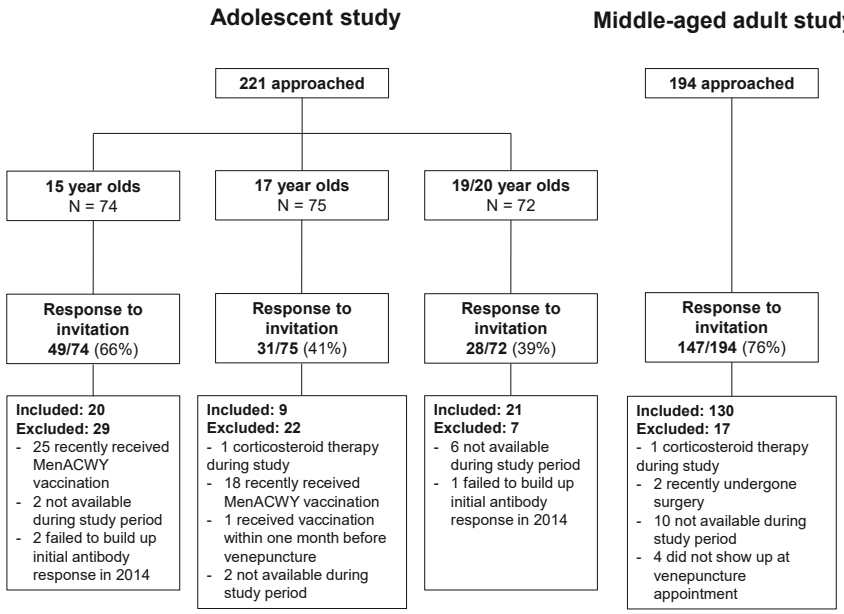

Figure 1. Flow-chart for response to invitation, inclusion and exclusion in the follow-up studies.

Persistence of antibodies after MenACWY-TT vaccination

Five years postvaccination, protective rSBA titers ≥8 were observed in 94% (MenC), 96% (MenW) and 94% (MenY) of the adolescents (Figure 2). Protection against all three serogroups was present in 88% of the participants.

Middle-aged adults showed rSBA titers ≥8 against MenC in 32%, against MenW in 65% and against MenY in 71% (Figure 2 and Table 1) at the five-year timepoint. Only 19% of the participants were still protected against all three serogroups after five years. The meningococcal specific GMTs differed significantly between the age groups not only at 1 month and 1 year but now also at five years after vaccination (Table 1). The proportion of adolescents showing rSBA titers above the more conservative threshold of ≥128 was significantly higher for all serogroups compared with the proportion of protected middle-aged adults (Table 1). A considerable difference was seen for MenC, as 88% of the adolescents showed rSBA titers ≥128 while only 13% of the middle-aged adults showed titers above this more conservative threshold (Table 1).

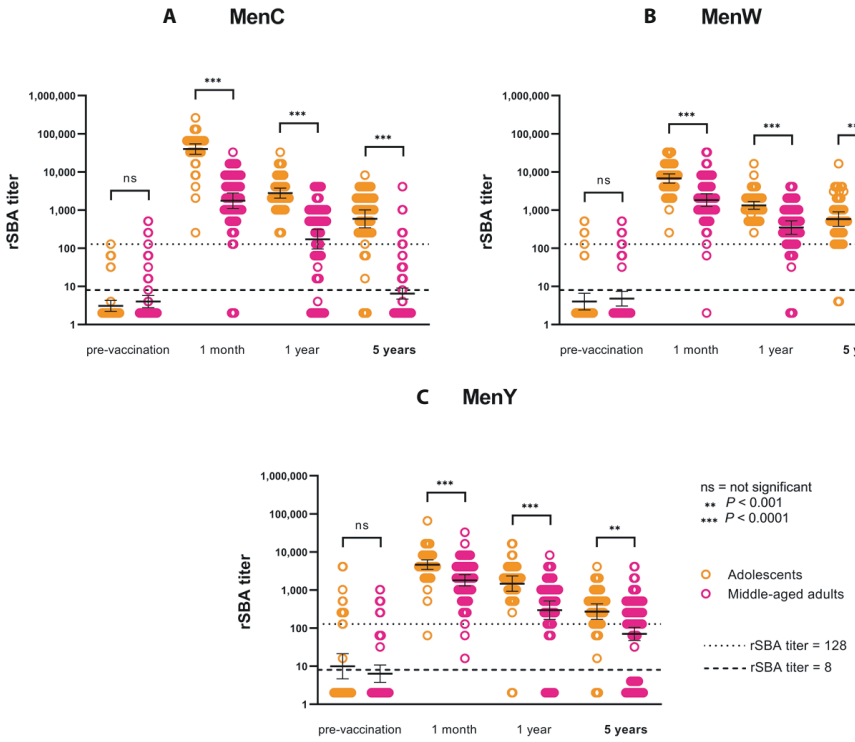

Figure 2. Longitudinal meningococcal serogroup C (**a**), W (**b**) and Y (**c**) specific geometric mean titers (GMTs) of serum bactericidal antibody with baby rabbit complement (rSBA) for two age groups at pre-vaccination, 1 month, 1 year, and 5 years after a meningococcal serogroup A, C, W, Y conjugated to tetanus toxoid (MenACWY-TT) vaccination. Error bars indicate 95% confidence intervals. The orange and pink dots represent the individual measured titers. *p*-values for the pre-vaccination timepoint and five-year timepoint were calculated with Mann–Whitney test. *p*-values for the 1 month and 1 year timepoint were calculated on natural log-transformed values with linear regression, adjusting for pre-vaccination titers.

Table 1. Meningococcal serogroup C, W, Y (MenCWY)-specific geometric mean rSBA titers (GMTs), MenACWY-polysaccharide (PS)-specific concentrations (GMCs), and proportions of participants with a serum bactericidal antibody (rSBA) titer ≥8 and ≥128 with corresponding 95% confidence intervals (CI) determined five years after vaccination.

Antibody		**Age group**		***p*-value**
		Adolescents (n = 50)	Middle-aged adults (n = 130)	
MenA	GMC MenA-PS-specific IgG µg/mL (95% CI)	1.8 (1.2–2.5)	2.8 (2.0–3.9)	0.1532
MenC	GMT (95% CI)	588 (341–1014)	6.5 (4.6–9.0)	<0.0001
	% rSBA-titer ≥8 (95% CI)	94% (82–98)	32% (24–40)	<0.0001
	% rSBA-titer ≥128 (95% CI)	88% (75–95)	13% (8–20)	<0.0001
	GMC MenC-PS-specific IgG µg/mL (95% CI)	10.1 (7.7–13.2)	1.3 (0.9–1.7)	<0.0001
MenW	GMT (95% CI)	578 (372–898)	52.5 (35.4–78.3)	<0.0001
	% rSBA-titer ≥8 (95% CI)	96% (85–99)	65% (56–73)	<0.0001
	% rSBA-titer ≥128 (95% CI)	96% (85–99)	56% (47–65)	<0.0001
	GMC MenW-PS-specific IgG µg/mL (95% CI)	1.3 (0.8–2.1)	0.5 (0.4–0.6)	0.0008
MenY	GMT (95% CI)	270 (170–430)	70.5 (47.2–105)	0.0002
	% rSBA-titer ≥8 (95% CI)	94% (82–98)	71% (62–79)	0.0006
	% rSBA-titer ≥128 (95% CI)	86% (73–94)	64% (55–72)	0.0036
	GMC MenY-PS-specific IgG µg/mL (95% CI)	2.2 (1.3–3.6)	0.9 (0.7–1.2)	0.0045

p-value proportions calculated with Fisher's exact test. *p*-value difference in GMT and GMC calculated with Mann–Whitney test. *p*-value difference in proportions calculated with Wilson score interval with continuity correction.

The adolescents showed significantly higher MenC-PS, MenW-PS, and MenY-PS specific serum IgG concentrations compared to the middle-aged adults, while, for MenA, no significant difference between the age groups was observed (Table 1 and Figure 3). While the GMTs of the three serogroups in adolescents were comparable or at most 2-fold higher, the GMC of MenC was almost 8-fold and 5-fold higher than the GMC of MenW and MenY, respectively.

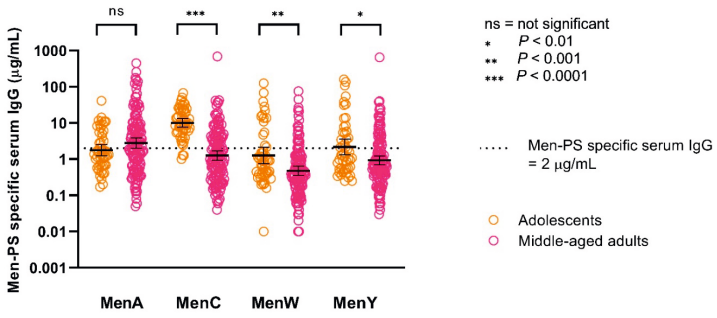

Figure 3. MenA, MenC-, MenW-, and MenY-PS specific serum IgG concentrations five years after a meningococcal serogroup A, C, W, Y conjugated to tetanus toxoid (MenACWY-TT) vaccination. Error bars indicate 95% confidence intervals. The orange and pink dots represent the individual measured concentrations. *p*-values were calculated using the Mann–Whitney test.

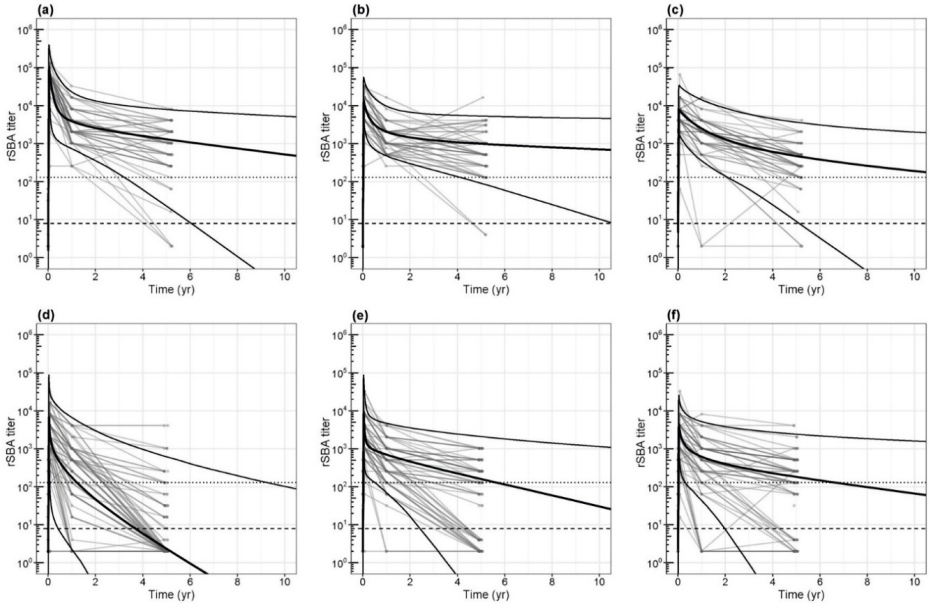

Figure 4. The predicted meningococcal rSBA titers for serogroup C (**a**) and (**d**), W (**b**) and (**e**) and Y (**c**) and (**f**) after a meningococcal serogroup A, C, W, Y conjugated to tetanus toxoid (MenACWY-TT) vaccination in adolescents (**a**)–(**c**) as a booster vaccination after being primed with a meningococcal serogroup C conjugated to tetanus toxoid (MenC-TT) vaccination at young age, and in middle-aged (**d**)–(**f**) adults as a primary vaccination, estimated by the bi-exponential decay model. Individual measurements are connected and presented as grey lines. Bold lines represent the 5% percentile, median, and 95% percentile rSBA titers. Dashed lines indicate correlate of protection (rSBA titer =8) and conservative threshold of protection (rSBA titer =128), respectively.

Waning of functional antibodies and duration of protection

In adolescents, the estimated median rSBA titers of all three serogroups, using the bi-exponential decay model, remained above the correlate of protection (rSBA ≥8) for 32, 98, and 33 years for MenC, MenW, and MenY, respectively (Figure 4 and Table 2). For MenC and MenY, it was estimated that the median rSBA titer would reach the more conservative threshold of ≥128 at 17 years and 13 years postvaccination, respectively (Figure 4), while the median MenW rSBA titer was estimated to remain above this threshold for 43 years. In contrast, the middle-aged adults showed a median rSBA titer below the threshold of 8 against MenC already after 3.7 years due to a steep decay in antibodies that continued after the first year postvaccination (Figure 4). The decay of MenW- and MenY-specific functional antibodies is less steep compared to the decay for MenC and median protection was estimated to continue up to 14 and 21 years after vaccination, respectively (Table 2). Waning of serogroup-specific functional antibodies continues over time and follows a pattern characterized by a rapid decay in the first year and a slower decay thereafter (Table 2, Figure 4). Between one year and five years after vaccination, mean annual decay rates vary from 0.58–1.65 except for MenC antibodies in middle-aged adults that still show a mean annual decay rate of 6.65 after the first year up to the fifth year, similar to the decay rate in the first year after vaccination (10.2).

Table 2. Fold changes and mean annual decay rates (relative decrease) in meningococcal serogroup C, W, Y (MenCWY)-specific geometric mean titers, and minimal (2.5% percentile) and median duration of protection (median rSBA titer ≥8).

Antibody		Adolescents	Middle-aged adults
MenC	Fold-change 1 month vs. 1 year	14.3	10.2
	Fold-change 1 year vs. 5 years	4.7	26.6
	Mean annual decay rate 1–5 years	1.18	6.65
	Minimal duration of protection	4.6 years	0.2 years
	Median duration of protection	32.4 years	3.7 years
MenW	Fold-change 1 month vs. 1 year	5.1	5.3
	Fold-change 1 year vs. 5 years	2.3	6.6
	Mean annual decay rate 1–5 years	0.58	1.65
	Minimal duration of protection	7.0 years	1.7 years
	Median duration of protection	97.7 years	13.9 years
MenY	Fold-change 1 month vs. 1 year	3.2	6.1
	Fold-change 1 year vs. 5 years	5.4	4.2
	Mean annual decay rate 1–5 years	1.35	1.05
	Minimal duration of protection	3.7 years	1.4 years
	Median duration of protection	33.4 years	20.8 years

Correlation between functional antibodies and serum IgG concentrations

Comparison of the functional antibody titers using the rSBA assay with serogroup-specific serum IgG concentrations measured by MIA demonstrated a good correlation of R = 0.88 for MenC in adolescents and R = 0.64 in middle-aged adults (Figure 5). The protected proportions for the rSBA and IgG (using the arbitrary IgG cut-off of ≥2 µg/mL) respectively are comparable for MenC in both adolescents (94% and 96%) and middle-aged adults (32% and 38%). However, 20 out of 89 (22%) of middle-aged adults with low bactericidal activity showed MenC-PS specific IgG concentrations that exceeded 2 µg/mL. A correlation of R = 0.55 was observed for MenW antibodies in adolescents, and, although adolescents showed sufficient killing against MenW in 96%, only 28% reached the MenW-PS specific IgG threshold of ≥2 µg/mL. In addition, in middle-aged adults, this discrepancy was observed, with protective titers in 65% while only 19% possessed a MenW-PS specific IgG concentration of 2 µg/mL or higher. The correlation was poor for MenY, especially in the middle-aged adults (R = 0.16).

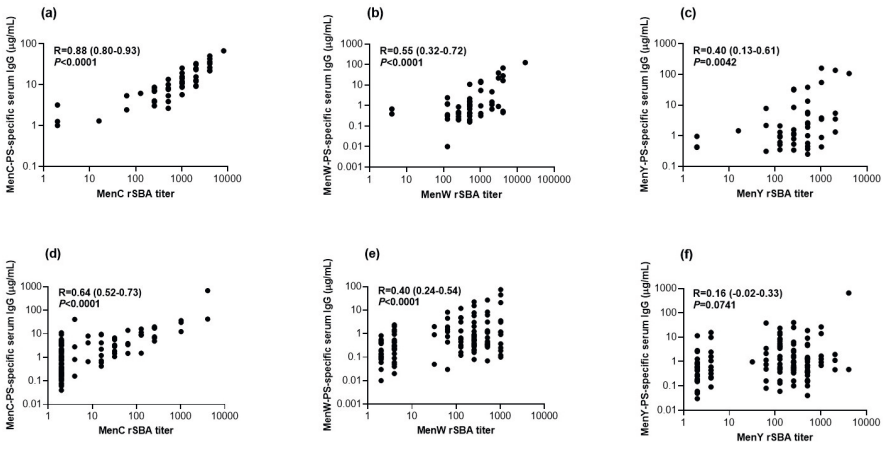

Figure 5. Correlation between the meningococcal serogroup C (**a**) and (**d**), W (**b**) and (**e**) and Y (**c**) and (**f**) polysaccharide (MenC, W, Y-PS) specific antibody concentrations and rSBA titers, five years after a meningococcal serogroup A, C, W, Y conjugated to tetanus toxoid (MenACWY-TT) vaccination, in both adolescents (**a**)–(**c**) and middle-aged adults (**d**)–(**f**). The correlations were analyzed using Spearman's rho correlation test.

DISCUSSION

In this study, we estimated the long-term protection against invasive meningococcal CWY disease after a single MenACWY-TT vaccination in MenC-vaccinated adolescents and in middle-aged adults and investigated the persistence of meningococcal serum antibodies in these two age groups. Five years after a single MenACWY-TT vaccination, the proportion of adolescents with a rSBA titer ≥8 for MenCWY is 88%. Based on rSBA titers, the median duration of protection in adolescents was estimated to be 32 years against MenC, 98 years against MenW and 33 years against MenY using a bi-exponential decay model. In contrast, in middle-aged adults who received the MenACWY-TT vaccination at 50–65 years of age, only 19% possessed protective MenCWY antibody titers five years postvaccination with an estimated median duration of protection of 4, 14, and 21 years against MenC, MenW and MenY, respectively.

Five years after vaccination adolescents showed significantly higher GMTs for the meningococcal serogroups CWY compared to middle-aged adults. This difference seems due to both significantly higher peak titers one month after vaccination for all three serogroups and a lower antibody decay rate for MenC and MenW in adolescents in the years thereafter. In former studies, adolescents showed a good immune response in reaction to a MenACWY-TT vaccination [14, 19-21, 29, 48, 49] and protection is known to last for at least several years [50-52]. Information about long-term protection in middle-aged adults is, however, scarce and the available studies had a short follow-up or investigated other conjugate vaccines or the plain polysaccharide vaccine [53-56]. The only comparable study was done by Borja-Tabora et al. [26], where seroprotection in adolescents and adults 18–55 years of age was also compared five years after a MenACWY-TT vaccination. While we found significantly longer protection in adolescents for all serogroups, they found mixed differences and a remarkable significantly higher protection level against MenY in adults. These differences might be at least partly explained by the younger age of the adults (18–55) than that of the middle-aged adults of 50–65 years in our study at time of vaccination.

When naïve B cells encounter a new antigen, for instance through vaccination, the differentiation into antibody-producing B-cells and memory B cells is induced [57]. However, the response to a new antigen such as following primary vaccination might be hampered at older age, as a consequence of immunological ageing [58, 59]. Antibody production is maintained either by long-lived plasma cells or a continuously active pool of memory B cells. At older age, the naïve B cell pool is limited and changes in the bone marrow affect the storage and survival of plasma cells [59]. This might ex-

plain the limited persistence of the antibody response and therefore shorter duration of protection after vaccination in the middle-aged adults compared to the adolescents, as described before [59].

The age groups in our study differed in vaccination history, with a MenC conjugate vaccination for all adolescents at young age while middle-aged adults did not receive a meningococcal vaccination earlier. This might have influenced the difference in adolescents and middle-aged adults with regard to MenC protection levels. However, van der Heiden et al. [18] observed a booster-like response for MenC in the Dutch middle-aged adult group after a first MenC conjugate vaccination. MenC-PS specific IgG concentrations increased within seven days while, for MenW and MenY, this early increase was not observed. Preexisting immunity for MenC is likely since a higher incidence of MenC disease was observed between 1998 and 2002 [12]. In the present study, it is possible that all age groups might have been exposed equally to MenC or MenW and have gained memory immunity. Adolescents in addition to natural exposure were vaccinated with MenC in early childhood. Since the mass-campaign in 2002 [60], only a very few cases of invasive meningococcal C disease occur every year in the Netherlands [61]. As a result, natural boosting for MenC after receiving the MenACWY-TT vaccination in 2014 is now very limited for our participants. We suggest that this absence of exposure might have contributed to the low MenC rSBA titers in the majority of middle-aged adults five years postvaccination and high annual decay rate of MenC IgG antibodies also in the 1–5 years postvaccination [5]. Our findings emphasize why natural boosting must be taken into account when designing vaccination strategies. Moreover, this is highlighted by our results that showed that the duration of protection in adolescents is three times longer for MenW (primary vaccination) compared to MenC (booster vaccination) and MenY (primary vaccination), possibly due to the recent MenW epidemic. Furthermore, factors related to the vaccine's profile might play a role in the differences between serogroups. In MenACWY-TT, the MenC (and also MenA) polysaccharide is conjugated indirectly to tetanus toxoid with an adipic dihydrazide, while MenW and MenY polysaccharide are directly conjugated to the carrier [62]. It is possible that this indirect conjugation improves SBA titers just after vaccination by optimizing outward presentation of the polysaccharide on the carrier protein to immune cells [63]. The effect of conjugation process on the long-term immune response remains, to the best of our knowledge, however unknown.

Remarkably, the MenC-PS specific IgG GMC in adolescents was almost 8-fold higher than for MenW five years after vaccination, while their rSBA GMTs were comparable. Because of the circulation of MenW, other non-capsular IgG antibodies or IgM anti-

bodies may contribute to the rSBA, while these are contributing less for MenC in the absence of recent circulation of this pathogen. As a result, the total meningococcal serum IgG concentration alone after a single vaccination might be less predictive for long-term seroprotection as defined by rSBA and, as such, the specific IgG after five years contributes only partly to the long-term protective titers. In contrast, when a strong IgG meningococcal antibody booster response is induced after revaccination or natural boosting, rSBA titers and IgG concentrations correlate better, as described earlier for a meningococcal booster vaccination [29].

The longitudinal aspect of this study with a follow-up of five years is an important strength of this study. Since the studies were performed in the same year, participants were exposed to the same natural circulation of meningococci so, when interpreting differences between age groups, timing can in that way be disregarded. Furthermore, we confirmed that median duration of protection after a meningococcal conjugate booster vaccination at adolescent age in Dutch adolescents is more than 30 years for MenC, which is in line with earlier findings by van Ravenhorst et al. [42]. Several limitations need to be considered such as the different meningococcal vaccination history in the two age groups and the large exclusion number in adolescents due to the mass campaign for MenACWY. It is worth mentioning that, within our adolescent group, three subgroups were present, vaccinated at age 10, 12, or 15 years. No clear differences in estimated duration of protection between these subgroups were observed in this study (data not shown), while, in these adolescents, significant differences were observed in serum bactericidal antibody titers between these subgroups in former studies [13, 14, 29]. The lack of a difference between these subgroups might be due to the low number of adolescents or to the long follow-up of five years. Furthermore, no historical rSBA data for MenA were available for the middle-aged adults and, therefore, we could not estimate long-term seroprotection for this serogroup. However, invasive MenA disease is very rare in the Netherlands [64, 65] and MenA-PS specific GMCs suggested adequate seroprotection in both age groups (Figure 3).

In conclusion, seroprotection for MenCWY is maintained in adolescents five years after a MenACWY-TT vaccination and estimated duration of protection is more than 30 years for MenC and MenY and even lifelong for MenW with a duration of 98 years. In contrast, middle-aged adults are insufficiently protected on the long run, especially against MenC, due to faster waning of antibodies. When vaccine-induced herd protection is established, natural boosting by meningococcal circulation will be diminished or even eradicated. This must be taken into account when vaccination strategies are adapted, to protect all age groups against invasive meningococcal disease.

ACKNOWLEGDEMENTS

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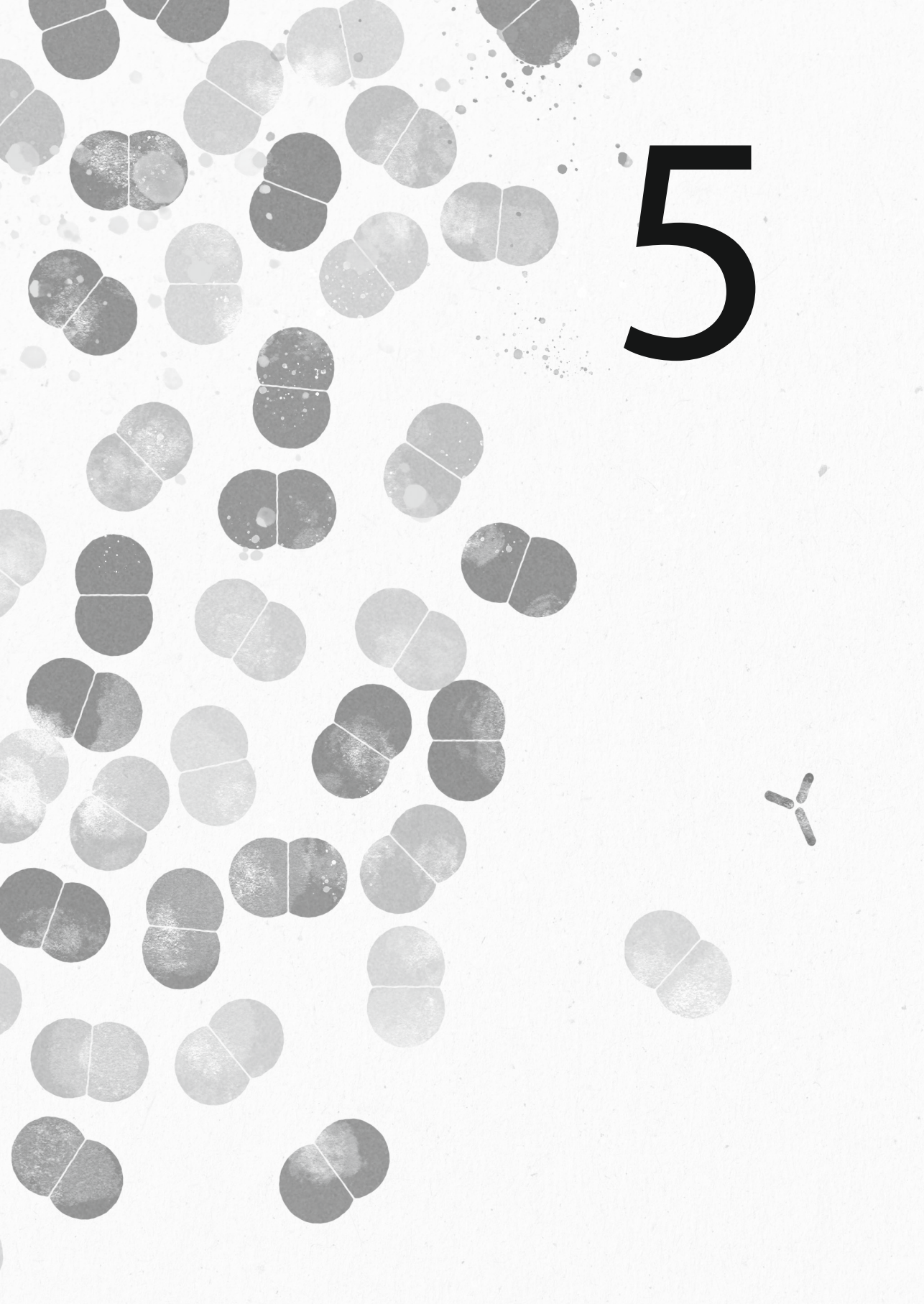

Sex-related differences in the immune response to meningococcal vaccinations during adolescence

Milou Ohm[1], Anna G.C. Boef[1], Susanne P. Stoof[1], Mariëtte B. van Ravenhorst[1], Fiona R.M. van der Klis[1], Guy A.M. Berbers[1], Mirjam J. Knol[1]

[1] Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands.

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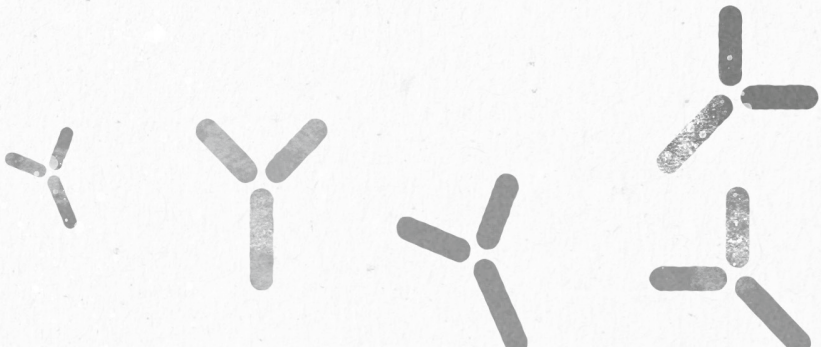

ABSTRACT

Background Immune responses to pediatric vaccinations have been reported to differ according to sex. Such sex-differential responses may become more pronounced during adolescence due to hormonal differences. We investigated whether the vaccine response following primary vaccination against meningococcal serogroup A (MenA), MenW and MenY and booster vaccination against MenC differed between girls and boys using data from two clinical studies.

Methods Children aged 10, 12 and 15 years, who had been primed with MenC vaccination between 14 months and 6 years of age, received a booster MenC vaccination or MenACWY vaccination. Polysaccharide-specific IgG concentrations and functional antibody titers (determined with the serum bactericidal antibody [SBA] assay) were measured at baseline, 1 month, 1 year, and 3 years (only MenC group) after vaccination. We calculated geometric mean concentrations and titers (GMC and GMT) ratios for girls vs. boys adjusted for age group. Additionally, we compared the proportion protected individuals between girls and boys at all timepoints.

Results This study included 342 girls and 327 boys from two clinical trials. While MenAWY antibody levels did not differ consistently 1 month after vaccination, all GMC- and GMT-ratios were in favor of girls 1 year after vaccination (range: 1.31 [1.02 to 1.70] for MenA IgG to 1.54 [1.10 to 2.16] for MenW IgG). Overall, MenC antibody levels were slightly higher in girls at all postvaccination timepoints (GMC- and GMT-ratios: 1.16/1.17 at one month, 1.16/1.22 at one year and 1.12/1.15 three years postvaccination). Higher MenC antibody levels were observed in 12- and 15-year-old girls compared to boys of the same age, whereas 10-year-old boys and girls had similar antibody levels. The percentage of participants protected (SBA titer ≥8) was very high (95 to 100%) at all timepoints, and did not differ significantly between boys and girls.

Conclusion Antibody responses were higher in girls than in boys for all serogroups at most timepoints after primary MenAWY vaccination and booster MenC vaccination. The differences in average titers were however small and the percentage participants with protective titers was very high for both sexes.

INTRODUCTION

Sex-related differences of genetic and hormonal nature are known to influence the immune system [1]. Biological factors related to sex, such as hormones, but also chromosomal differences are considered important in both infectious diseases and autoimmunity [2]. Invasive meningococcal disease (IMD) is a severe disease, caused by the Gram-negative bacterium *Neisseria meningitidis* [3], which can be prevented by vaccination. A meta-analytic evaluation of sex differences in IMD rates by age group in 10 countries found excess incidence rates in young males, but a reversed sex ratio in older adults with higher rates in females [4]. During a recent IMD-W outbreak in the Netherlands, females were affected more often than males (66% versus 34% respectively), although cases predominantly occurred in (older) adults [5]. Mortality data from New York City showed higher case fatality rates for IMD in females across all ages [6]. However, there is insufficient knowledge about the vaccine response at different stages of life in relation to sex and a paucity of clinical (vaccine) trials that include data analyzed by sex [7, 8]. Immune responses to several infant vaccinations have been reported to differ according to sex [9]. Such sex-differential responses may become more pronounced during adolescence due to hormonal differences. For example, while IgG and IgM levels are generally equal between the sexes pre-puberty, these immunoglobulins are higher in females post-puberty [2]. Knowledge on sex differences in vaccine response could contribute to the rationale of vaccine strategies, as was previously proposed for influenza vaccination [10].

A meningococcal serogroup C (MenC) conjugate vaccine was introduced in the national immunization programme (NIP) in the Netherlands in September 2002 for 14-month-olds [11]; children born from July 2001 onwards were therefore eligible for vaccination. Furthermore, a catch-up campaign for children up to 18 years of age (born from June 1983 until July 2001) was conducted from June until November 2002 [11]. Recently, the MenC conjugate vaccine was replaced by a meningococcal serogroup A, C, W and Y (MenACWY) conjugate vaccine in response to an increase of IMD serogroup W (IMD-W) [12]. During this increase, teenagers were the main target population for vaccination, since they were disproportionally affected during this increase [13] and since this age group has the highest meningococcal carriage rate [14]. A mass campaign for 14-18 year-olds (born between January 2001 and December 2005) was conducted, and all 14-year-olds are now offered a MenACWY-TT booster dose, after priming at the age of 14 months. Data on protection levels after meningococcal vaccination separated by sex are scarce and lacking for adolescents in particular.

Our objective was to explore the sex-related differences in the immune response following adolescent meningococcal vaccination in two clinical studies that were carried out between 2011–14 and 2015–19. We determined the quantity and functionality of serum and salivary MenACWY antibody levels in individuals aged 10, 12 and 15 years at time of vaccination, and assessed differences between the sexes.

METHODS

Study populations

Two phase-IV clinical trials (clinical trial numbers: NL3372 and NL4286) enrolled participants in 2011 and 2014 to receive a MenC-TT or MenACWY-TT vaccine respectively at the age of 10, 12 or 15 years after being primed at young age (aged between 14 months and 6 years) with a MenC-TT vaccine, as previously described [15-17]. Serum samples were collected at baseline (T0), 1 month (T1) and 1 year (T2) after vaccination. In addition, from a subset of participants serum samples were collected at 3 years (T3) postvaccination (MenC booster vaccination group) [18].

Serological analyses

MenA-, MenC-, MenW-, and MenY polysaccharide (PS)-specific serum IgG, serum IgA and salivary IgA concentrations and tetanus toxoid (TT)-specific serum IgG concentrations were measured using a fluorescent-bead-based multiplex immunoassay (MIA) [19-22]. Functional antibodies were assessed with the serum bactericidal antibody (rSBA) assay using baby rabbit complement and MenA strain 3125, MenC strain C11 [23], MenW strain MP01240070 and MenY strain S-1975 as target strains. The correlate of protection (internationally accepted) of rSBA titer ≥8 was used for analyses, with the bactericidal titer defined as the dilution of the serum that corresponded with ≥50% killing after 60 minutes incubation [24-26]. When the titer fell below the cut-off of the assay (titer <4), a value of 2 was assigned.

Statistical analysis

The statistical analyses were performed using Excel, GraphPad Prism 8 and SPSS Statistics v24. Geometric mean concentrations (GMCs) of MenACWY-PS-specific IgG and TT-specific IgG and geometric mean titers (GMTs) for serogroup-specific SBA titers were calculated for girls and boys separately (across age groups) at T1 (1 month after booster vaccination) and T2 (1 year after booster vaccination). We used a generalized linear model to perform regression analyses per serogroup, using ln-transformed IgG levels or SBA titer at T1 or T2 as dependent variable and sex as independent variable. The

exponentiated regression coefficient for sex was used to obtain IgG GMC ratios or SBA GMT ratios for girls versus boys for each serogroup. We performed the MenAWY analyses (1) adjusted for age group and (2) adjusted for both age group and IgG or SBA at T0. For meningococcal serogroup A, W and Y, we did not perform separate analyses for the different age groups because of the small sample sizes. We performed MenC analyses (1) adjusted for study-group (8 groups which differed on the following aspects: booster age, priming age and MenC-TT or MenACWY-TT booster vaccination) and (2) adjusted for both study-group and IgG or SBA result at T0. We performed analyses per booster-age-group (10 years, 12 years and 15 years), and overall for each timepoint. Analyses were performed for an additional timepoint (T3: 3 years after MenC booster vaccination) for the subgroup for whom measurements at this additional timepoint were available. In addition, the proportion of protected (SBA titer ≥8) girls and boys at the different time points for each serogroup were compared by a Fischer's exact test. For serum IgA and salivary IgA we performed the same analyses as for IgG and SBA. No measurements at 3 years after booster vaccination were available for serum or salivary IgA. The same analyses were also performed for TT-specific serum IgG for the MenC booster vaccination group, with measurements available at baseline, one month and one year after vaccination. A p-value of <0.05 was considered as statistically significant.

RESULTS

Population characteristics

As shown in Table 1A, the distribution of girls and boys slightly differed across age groups in the study population for meningococcal serogroup A, W and Y with more girls in the youngest age group and more boys in the older age groups. Baseline IgG levels against meningococcal serogroups A, W and Y were generally low for both sexes. The percentage with protective SBA titers at baseline was similar for girls and boys for all three serogroups, with overall 20, 15, and 31% of the participants protected for serogroup A, W, and Y, respectively.

The characteristics of the study population for meningococcal serogroup C are described in Table 1B. Both the baseline MenC IgG concentrations and the percentage with protective SBA titers at baseline did not differ between girls and boys. The overall percentage of participants with protective SBA titers at baseline ranged from 10% among 12-year olds who were primed at 14 months of age, to 45% in 15-year olds who were primed at 6 years of age.

Table 1A. Characteristics of the study population for meningococcal serogroups A, W and Y.

Characteristic	Girls (n=121)	Boys (n=116)
Age group, n (%)		
10y	47 (38.8)	33 (28.4)
12y	36 (29.8)	43 (37.1)
15y	38 (31.4)	40 (34.5)
Baseline IgG in µg/mL, median (IQR)		
MenA	0.55 (0.29-1.39) *(n=118)*	0.44 (0.25-0.85) *(n=114)*
MenW	0.12 (0.05-0.44) *(n=119)*	0.08 (0.04-0.23) *(n=115)*
MenY	0.06 (0.03-0.12) *(n=119)*	0.05 (0.03-0.08) *(n=115)*
Baseline SBA titer		
MenA, median (range)	2 (2-2048)	2 (2-1024)
MenA ≥8, n(%)	24/117 (21)	22/115 (19)
MenW, median (range)	2 (2-512)	2 (2-2048)
MenW ≥8, n(%)	18/118 (15)	18/115 (16)
MenY, median (range)	2 (2-4096)	2 (2-4096)
MenY ≥8, n(%)	35/117 (30)	38/115 (33)

Abbreviations: MenA, meningococcal serogroup A; MenW, meningococcal serogroup W135; MenY, meningococcal serogroup Y; IgG immunoglobulin G; SBA, serum bactericidal antibody; IQR, interquartile range.

Meningococcal serogroups A, W and Y: IgG and SBA

The IgG GMCs and SBA GMTs for MenA, MenW and MenY for girls and boys (across age groups) at 1 month and 1 year after booster vaccination, and the corresponding (adjusted) GMC ratios and GMT ratios are shown in Table 2A. At 1 month after the MenACWY vaccination, IgG levels and SBA titers did not differ consistently between sexes, as shown in Figure 1. Adjustment for IgG level/SBA titer at baseline slightly changed some estimates, but did not alter the observed trend. At 1 year after vaccination, all GMC/GMT ratio estimates were in favor of girls; ratio estimates ranged from 1.31 (1.02 to 1.70) for MenA IgG to 1.54 (1.10 to 2.16) for MenW IgG. Estimates were somewhat attenuated after adjusting for IgG/SBA at T0, e.g. to 1.18 (0.95 to 1.47) and 1.40 (1.00 to 1.94) respectively for the previously mentioned GMC ratios for MenA and MenW IgG.

Table 1B Characteristics of the study population for meningococcal serogroups C.

Characteristic				Girls (n=342)	Boys (n=327)
Group*, n (%)					
10y	MenC-TT		14m	53 (15.5)	38 (11.6)
10y	MenACWY-TT		14m	47 (13.7)	33 (10.1)
12y	MenC-TT		3y	44 (12.9)	47 (14.4)
12y	MenC-TT		14m	37 (10.8)	45 (13.8)
12y	MenACWY-TT		14m	36 (10.5)	43 (13.2)
15y	MenC-TT		6y	41 (12.0)	45 (13.8)
15y	MenC-TT		3y	46 (13.5)	36 (11.0)
15y	MenACWY-TT		3y	38 (11.1)	40 (12.2)
Baseline IgG in µg/mL, median (IQR)					
MenC					
Overall				0.26 (0.15-0.51) *(n=338)*	0.24 (0.14-0.46) *(n=326)*
10y			14m	0.21 (0.12-0.43) *(n=98)*	0.27 (0.13-0.53) *(n=70)*
12y			3y	0.24 (0.15-0.66) *(n=44)*	0.26 (0.18-0.47) *(n=47)*
12y			14m	0.21 (0.10-0.43) *(n=71)*	0.21 (0.11-0.43) *(n=88)*
15y			6y	0.45 (0.28-0.83) *(n=41)*	0.25 (0.16-0.52) *(n=45)*
15y			3y	0.28 (0.18-0.51) *(n=84)*	0.24 (0.14-0.46) *(n=76)*
Baseline SBA titer					
MenC, median (range)					
Overall				2 (2-16384)	2 (2-16384)
10y			14m	2 (2-2048)	2 (2-512)
12y			3y	2 (2-3072)	2 (2-4096)
12y			14m	2 (2-1024)	2 (2-2048)
15y			6y	4 (2-16384)	2 (2-768)
15y			3y	2 (2-2048)	2 (2-16384)
MenC ≥8, n(%)					
Overall				66/337 (19.6)	70/326 (21.5)
10y		14m		12/98 (12)	13/70 (19)
12y		3y		14/44 (32)	17/47 (36)
12y		14m		6/71 (9)	10/88 (11)
15y		6y		20/41 (49)	19/45 (42)
15y		3y		14/83 (17)	11/76 (15)

Abbreviations: MenC, meningococcal serogroup C; IgG immunoglobulin G; SBA, serum bactericidal antibody; IQR, interquartile range.

*groups differed on the following aspects: 1) booster age, 2) MenC-TT or MenACWY-TT booster vaccination and 3) priming age.

Meningococcal serogroup C: IgG and SBA

For MenC, IgG GMCs and SBA GMTs are shown in Table 2B. Overall, both IgG and SBA were higher in girls at all postvaccination timepoints (Figure 2), e.g. at 1-month after the booster the overall IgG GMC ratio was 1.16 (1.02-1.31) and the overall SBA GMT ratio was 1.17 (1.01-1.35). When separated by age group, higher MenC IgG levels and SBA titers were observed in 12-and 15-year-old girls than in boys, whereas 10-year-old boys and girls had similar IgG levels and SBA titers.

Table 2A. Geometric mean IgG concentrations and geometric mean SBA titers for girls and boys and geometric mean concentration/titer ratios for girls versus boys for meningococcal serogroups A, W and Y at 1 month and 1 year following MenACWY-TT vaccination.

	Girls (n=121)			Boys (n=116)			GMC ratio (95% CI)		GMT ratio (95% CI)	
	n	GMC (95% CI)	GMT (95%CI)	n	GMC (95% CI)	GMT (95% CI)	adjusted for age group	adjusted for age group & IgG at T0*	adjusted for age group	adjusted for age group & SBA at T0*
MenA										
T1	119	25.6 (20.4-32.2)	3482 (2614-4638)	113	28.5 (22.7-35.7)	4600 (4045-5232)	0.93 (0.68-1.27)	0.85 (0.63-1.15)	0.80 (0.59-1.09)	0.79 (0.57-1.08)
T2	116	7.55 (6.28-9.08)	914 (711-1176)	111	5.77 (4.78-6.96)	649 (506-832)	**1.31 (1.02-1.70)**	1.18 (0.95-1.47)	**1.47 (1.04-2.08)**	**1.41 (1.00-1.99)**
MenW										
T1	119	5.11 (3.80-6.88)	5449 (3982-7456)	113	4.69 (3.51-6.27)	6267 (5377-7305)	1.20 (0.81-1.77)	1.04 (0.71-1.53)	0.93 (0.66-1.31)	0.92 (0.65-1.29)
T2	116[a]	3.91 (3.00-5.10)	1311 (1026-1676)	111	2.62 (2.10-3.27)	1041 (892-1214)	**1.54 (1.10-2.16)**	**1.40 (1.00-1.94)**	**1.34 (1.01-1.77)**	**1.34 (1.01-1.76)**
MenY										
T1	119	5.98 (4.55-7.85)	4408 (3510-5534)	113	5.60 (4.27-7.33)	3535 (3055-4091)	1.12 (0.77-1.63)	1.07 (0.76-1.51)	**1.32 (1.02-1.72)**	**1.35 (1.05-1.75)**
T2	116	1.98 (1.48-2.64)	1501 (1134-1986)	111	1.40 (1.03-1.92)	1183 (955-1466)	1.47 (0.97-2.23)	1.39 (0.97-2.01)	1.34 (0.95-1.90)	1.37 (0.98-1.92)

Abbreviations: MenA, meningococcal serogroup A; MenW, meningococcal serogroup W135; MenY, meningococcal serogroup Y; IgG immunoglobulin G; SBA, serum bactericidal antibody; GMC, geometric mean concentration; GMT, geometric mean titer; CI, confidence interval; T0, before vaccination; T1, 1 month after vaccination; T2, 1 year after vaccination. Significant results ($p<0.05$) are outlined in bold.

*Number of girls (F) and boys (M) excluded from the analysis due to missing IgG/SBA at T0: MenA IgG T1: 2F, 1M; MenA IgG T2: 2F, 1M; MenW IgG T1: 1F; MenW IgG T2: 1F; MenY IgG T1: 1F; MenY IgG T2: 1F; MenA SBA T1: 3F; MenA SBA T2: 3F; MenW SBA T1: 2F; MenW SBA T2: 2F; MenY SBA T1: 3F; MenY SBA T2: 3F.

[a]*Number of girls included in the GMT: n=115 (one missing SBA).*

Table 2B. Geometric mean IgG concentrations and geometric mean SBA titers for girls and boys and geometric mean concentration/titer ratios for girls versus boys for meningococcal serogroup C at 1 month, 1 year and 3 years following MenC-TT/MenACWY-TT booster vaccination.

MenC	Girls (n=121)				Boys (n=116)				GMC ratio (95% CI)		GMT ratio (95% CI)	
	n (IgG)	n (SBA)	GMC (95%CI)	GMT (95%CI)	n (IgG)	n (SBA)	GMC (95%CI)	GMT (95%CI)	adjusted for study group	adjusted for study group & IgG at T0*	adjusted for study group	adjusted for study group & SBA at T0*
T1												
10y	97	97	124 (98.6-156)	29012 (21723-38748)	68	68	124 (103-150)	29079 (23331-36244)	1.00 (0.74-1.37)	0.99 (0.73-1.35)	1.00 (0.68-1.47)	0.99 (0.67-1.45)
12y	113	112	191 (167-219)	41167 (35033-48374)	133	133	160 (142-181)	34586 (30143-39683)	1.19 (0.99-1.42)	1.19 (0.99-1.42)	1.18 (0.96-1.45)	1.18 (0.97-1.45)
15y	122	121	184 (162-209)	43726 (38179-50078)	121	121	147 (128-169)	34428 (29264-40503)	**1.24 (1.03-1.49)**	**1.25 (1.04-1.51)**	**1.27 (1.04-1.56)**	**1.30 (1.06-1.59)**
overall	332	330	166 (151-183)	37973 (33914-42519)	322	322	147 (136-160)	33285 (30276-36592)	**1.16 (1.02-1.31)**	**1.15 (1.02-1.30)**	**1.17 (1.01-1.35)**	**1.17 (1.01-1.35)**
T2												
10y	93	93	9.73 (7.91-12.0)	1523 (1159-2002)	66	66	9.66 (7.77-12.0)	1581 (1217-2054)	1.02 (0.76-1.36)	1.02 (0.77-1.35)	0.97 (0.67-1.42)	0.97 (0.67-1.41)
12y	111	110	17.5 (14.7-20.7)	3355 (2755-4085)	132	132	13.7 (11.5-16.2)	2383 (1994-2848)	**1.26 (1.01-1.57)**	**1.26 (1.03-1.55)**	**1.38 (1.08-1.77)**	**1.39 (1.10-1.77)**
15y	121	121	25.6 (22.0-29.9)	4780 (4007-5701)	117	117	22.3 (19.0-26.1)	3871 (3221-4651)	1.16 (0.94-1.42)	1.08 (0.89-1.33)	1.24 (0.98-1.58)	1.24 (0.98-1.57)
overall	325	324	17.0 (15.3-19.0)	3052 (2677-3480)	315	315	15.2 (13.7-17.0)	2619 (2323-2952)	**1.16 (1.01-1.32)**	1.12 (0.99-1.28)	**1.22 (1.04-1.43)**	**1.22 (1.04-1.42)**
T3												
10y	40	40	6.27 (4.82-8.16)	578 (404-827)	26	26	6.62 (5.22-8.40)	686 (533-884)	0.95 (0.66-1.36)	1.09 (0.75-1.59)	0.84 (0.53-1.34)	0.92 (0.57-1.49)
12y	37	37	15.3 (12.1-19.5)	2335 (1670-3264)	38	38	12.4 (8.54-17.9)	1707 (1202-2422)	1.24 (0.81-1.89)	1.26 (0.85-1.87)	1.37 (0.86-2.17)	1.43 (0.92-2.20)
15y	33	33	20.7 (15.9-27.0)	4096 (2994-5603)	25	25	17.5 (13.6-22.5)	3191 (2373-4293)	1.19 (0.83-1.69)	1.07 (0.76-1.50)	1.28 (0.85-1.95)	1.27 (0.84-1.93)
overall	110	110	12.1 (10.2-14.4)	1663 (1300-2129)	89	89	11.4 (9.32-13.8)	1559 (1253-1940)	1.12 (0.90-1.41)	1.14 (0.92-1.41)	1.15 (0.88-1.50)	1.18 (0.91-1.53)

Abbreviations: MenC, meningococcal serogroup C; IgG immunoglobulin G; SBA, serum bactericidal antibody; GMC, geometric mean concentration; GMT, geometric mean titer; CI, confidence interval; T0, before vaccination; T1, 1 month after vaccination; T2, 1 year after vaccination; T3 3 years after vaccination. Significant results ($p<0.05$) are outlined in bold. *Number of girls (F) and boys (M) excluded from the analysis due to missing IgG/SBA at T0: IgG T1 10y: 1F; IgG T1 overall: 1F; IgG T2 10y: 1F IgG T2 overall: 1F; SBA T1 10y: 1F; SBA T1 15y: 1F; SBA T1 overall: 2F; SBA T2 10y: 1F; SBA T2 15y: 1F; SBA T2 overall: 2F.

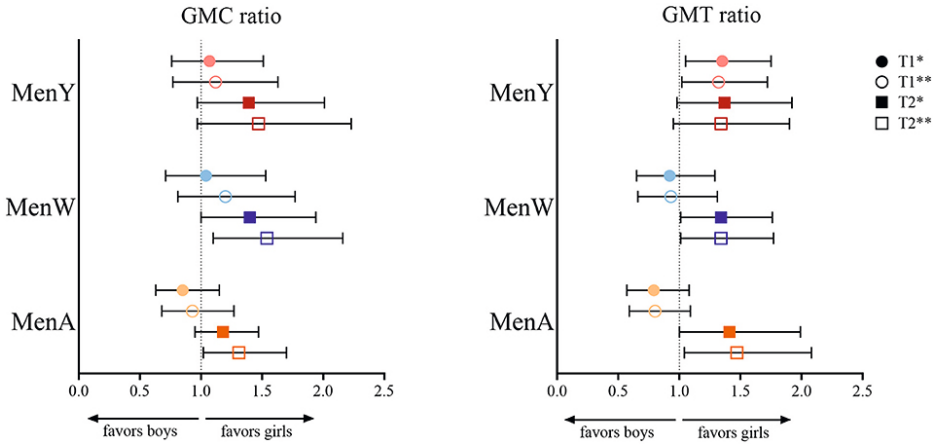

Figure 1. Geometric mean concentration (GMC) ratio and geometric mean titer (GMT) ratio for meningococcal serogroup A (MenA), MenW and MenY in girls versus boys at one month (T1) and one year (T2) after a meningococcal serogroup A, C, W and Y conjugated to tetanus toxoid (MenACWY-TT) vaccine in adolescents who were primed at young age (aged between 14 months and 6 years) with a MenC-TT vaccine. *adjusted for age group and baseline level at T0 (IgG or SBA respectively for GMC and GMT ratio) ; **adjusted for age group

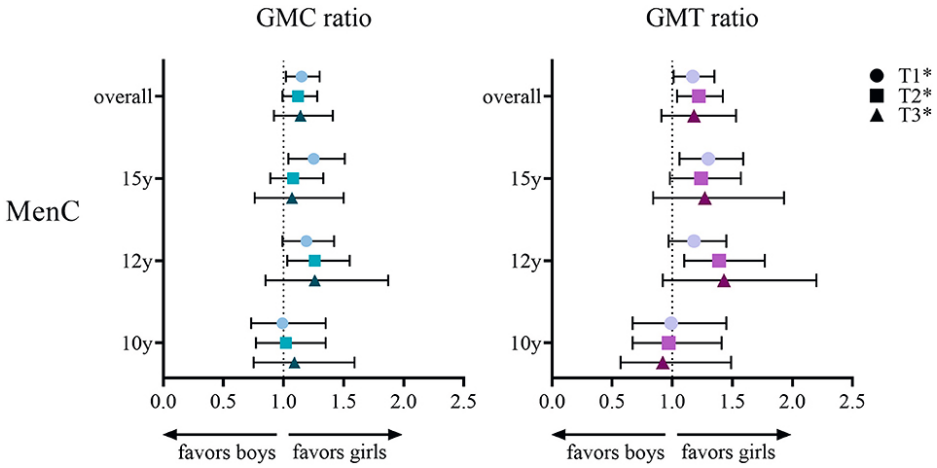

Figure 2. Geometric mean concentration (GMC) ratio and geometric mean titer (GMT) ratio for meningococcal serogroup C (MenC) in girls vs. boys per age group (10, 12 or 15 years) and overall at 1 month (T1), 1 year (T2) and 3 years (T3) after either a meningococcal serogroup A, C, W and Y conjugated to tetanus toxoid (MenACWY-TT) vaccine or a MenC-TT vaccine in adolescents who were primed at young age (aged between 14 months and 6 years) with a MenC-TT vaccine. *adjusted for age group and baseline level at T0 (IgG or SBA respectively for GMC and GMT ratio).

Meningococcal serogroup A, C, W and Y: proportions protected

The vast majority of participants (96-100%), both girls and boys, were protected against all serogroups 1 month and 1 year after vaccination. There were no significant differences in the proportions protected (SBA ≥8) between girls and boys at any timepoint or for any serogroup (Table 3). Three years after vaccination, all girls and boys (n=110 and n=89 respectively) were still protected against MenC [18].

Table 3. Proportions protected according to SBA titer for girls and boys for all serogroups and timepoints.

Serogroup	Timepoint	Girls			Boys			p-value*
		N protected (SBA ≥8)	N totaal	%	N protected (SBA≥8)	N totaal	%	
A	1 mo	115	119	96.6	113	113	100	0.122
	1 yr	112	116	96.6	107	111	96.4	1.000
W	1 mo	115	119	96.6	113	113	100	0.122
	1 yr	112	115	97.4	111	111	100	0.247
Y	1 mo	118	119	99.2	113	113	100	1.000
	1 yr	112	116	96.6	110	111	99.1	0.370
C	1 mo	329	330	99.7	322	322	100	1.000
	1 yr	322	324	99.4	315	315	100	0.499
	3 yr[a]	110	110	100	89	89	100	NA

Abbreviations: mo = month, yr= year, NA = not applicable.

*p-values (two-sided) of the difference in proportion protected between girls and boys were determined with Fisher's exact test

[a]determined in a subgroup of participants who participated in a follow-up study

Serum and salivary meningococcal IgA

Results for serum IgA and salivary IgA are shown in Supplementary Tables 2A,B (with baseline characteristics in Supplementary Tables 1A,B). The observed trend of the IgA results was similar to IgG and SBA, either showing no clear difference or somewhat higher levels in girls. However, although the trend was similar, the difference only reached significance for MenY serum IgA at T2 when adjusted for age group only (p=0.037) or for age group and baseline levels (p=0.019). For MenC, a significant difference towards girls was observed for serum IgA at 1 year after vaccination in 12-year-olds, when adjusted for age and baseline level. A significant difference was found for MenC salivary IgA at 1 month after vaccination in 12-year-olds as well as for the overall group.

Serum tetanus IgG

Results for TT-specific serum IgG were only available for the MenC-booster group for baseline, 1 month and 1 year after vaccination (Supplementary Table 3). We found a

significant difference for 10-year-olds at T2 with a higher level in boys (GMC ratio girls versus boys: 0.72 [0.54-0.96], p=0.024), but we found no significant difference in other age groups nor at other timepoints.

DISCUSSION

In this study, we evaluated sex-related differences in the immune response to a meningococcal conjugate vaccine in adolescents. We found slightly higher antibody levels in girls than in boys at the age of 12 or 15 years respectively, and at more than a month after vaccination. Our results suggest some sex-based disparity in the meningococcal vaccine-induced immune response during adolescence. Since this is a period characterized by a developing and changing hormonal system while simultaneously being prone for carriage of meningococci, a sufficient vaccine response is important.

To our knowledge, we are the first to report meningococcal vaccine-induced sex-specific immune responses in adolescents. A meta-analysis by Voysey et al. found consistently higher immune responses in girls than boys - all aged younger than 3 years - to a (diphtheria cross-reacting material [CRM197] conjugated) meningococcal ACWY vaccine for serogroup A, W and Y, but not for serogroup C with most geometric mean MenC ratios close to 1 [9]. This is in contrast to our results that showed favorable results in girls for all serogroups including serogroup C, albeit not for each timepoint. In line with our findings, a study that investigated the vaccine response to other capsular conjugate vaccines like the pneumococcal vaccine and Haemophilus influenza type B (Hib) vaccine reported no differences or higher antibody levels in females, although they included infants and young children [27]. Similar, a trend of comparable or higher tetanus antibody levels in boys was also observed in that study.

It was previously proposed that the carrier protein in conjugate vaccines might have a sex-differential effect [9, 28]. In the current study, all participants received a meningococcal vaccine conjugated to tetanus toxoid and we could not make a comparison between different carrier proteins. Yet, with regard to the carrier protein itself, we only identified a significant difference in 10-year-olds with higher tetanus antibody levels in boys rather than girls. This finding does not prove nor exclude a sex-differential effect towards females as promoted by the carrier protein.

Since age is inextricably linked with sex hormones - which induce variation of the immune profile during life - the influence of age should always be considered in stud-

ies comparing responses according to sex. Generally, estrogens have a variable (mostly activating) effect on the immune function, while progesterone is considered as a modulator or suppressive hormone and testosterone mainly acts as immunosuppressor [2, 29]. In adolescence, the actions of steroid hormones result in extensive changes to an individual's body [30], including the immune system. Therefore, our results cannot be translated directly to younger children or elderly, in whom sex-differences are hypothesized to be minimized due to the life-course related changing hormonal status. For instance, the effect of sex could be limited in postmenopausal females due to relatively high levels of progesterone compared to earlier in life (and comparable progesterone levels to males at older age), though genetic differences continue to exist. This is also highlighted by the fact that we found sex-differences in 12- and 15-year-olds but not in 10-year-olds. At the age of 10, most children are in a phase prior to, or at the start of the pubertal rise of reproductive hormones that is called the gonadarche [31, 32]. Before this phase, the effect of gonadal steroids on the vaccine response is expected to be limited. The implications of sex-differential effects for vaccination policy are therefore dependent on many factors and sex should always be considered in relation to age.

Not only vaccination or disease, but also asymptomatic carriage can induce the production of antibodies [33, 34]. We cannot exclude that carriage of the bacterium might have influenced our results, since serogroup C, W and Y are still prevalent in the Netherlands [13]. To what extent carriage might have affected our results remains uncertain, but evidence for sex-differential meningococcal carriage rates is limited. A large carriage study in the UK that investigated predisposing factors for meningococcal carriage in teenagers did not find an association between carriage and sex [35], similar to results from a study in adolescents in Australia [36]. In university students in the United States, meningococcal carriage was in fact associated with being male [37]. In this study, we did not find any significant sex-related differences in IgA levels. IgA is the dominant Ig type in mucosal tissues and thus important in the first line of defense at the location of carriage, e.g. the nasopharynx and its mucosal surfaces [38, 39]. Moreover, the sex-related differences in IgG levels we found were present after vaccination, but not before vaccination and carriage levels are known to increase after the age of 15 years [14]. Therefore, it appears unlikely that our results were confounded by naturally-acquired immunity.

In spite of a difference in geometric mean antibody levels, protection levels did not differ significantly up to 3 years postvaccination. Longer follow-up studies are necessary to investigate the implications for adolescents when antibodies wane. Although there seems to be a tendency of faster waning for MenAWY in males (with sex differ-

ences increasing over time), we did not observe this pattern for MenC. We have some data available 5 years after the MenACWY vaccination for a subgroup of participants, but these results were inconclusive due to small sample sizes and proportions protected were still very high among adolescents as was previously published [40]. We encourage future clinical trials, carriage studies but also serosurveillance studies - that often cover longer periods after vaccination due to the nature of the study - to report data stratified by sex. Thereby, the knowledge on sex differences in vaccine-induced immune responses could be expanded, not only for meningococci but also for other vaccine-preventable pathogens. Meningococcal vaccination policy might not change when long-term data would become available, which is supported by our finding that differences between sexes are limited 3 years postvaccination and protection levels at that timepoint were very high for both sexes. However, it might be relevant for other vaccine-preventable diseases if vaccine-induced immunity wanes fast in one sex but not the other.

One of the strengths of this study is the clinical trial setting of the studies with a fairly equal number of included boys and girls enabled *post-hoc* analysis without risk of selection bias. We investigated both IgG concentrations and functional antibody titers, which enabled analysis of the proportions protected in addition to geometric means of antibody levels. However, despite our trial has a follow-up time of three years, we found very high levels of protected participants at this latest timepoint. This hampered the exploration of clinical relevance of the biological differences that we found. Future modeling studies could estimate potential differences in duration of protection and serosurveillance studies should also consider presenting data by sex to explore sex-based differences in antibody levels across the population. One of the limitations of the study is the lack of information about every individual's pubertal maturation status at time of the study. Since the onset of puberty differs per individual, we could not analyse the results per puberty stage (pre-puberty vs. puberty) in addition to the age-specific analyses that we did. Furthermore, we could not analyse the MenAWY results per age group due to the limited number of participants in the MenACWY booster group. Nevertheless, we did have a large sample size in the MenC booster group which enabled us to examine MenC IgG, IgA and TT results per age group.

To conclude, our data showed that the vaccine responses following an adolescent MenC or MenACWY vaccination were slightly higher in 12- and 15-year-old girls than in boys. However, the percentage with protective titers was very high for both boys and girls. More research is needed to establish whether these findings are of clinical relevance on the long-term when antibodies wane and protection levels decrease.

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Supplementary Table 1A. Characteristics of the study population for meningococcal serogroups A, W and Y (IgA analyses).

Characteristic	Girls (n=121)	Boys (n=116)
Baseline serum IgA in µg/mL, median (IQR)		
MenA	0.075 (0.039-0.160) *(n=114)*	0.064 (0.032-0.115) *(n=113)*
MenW	0.017 (0.007-0.046) *(n=114)*	0.019 (0.001-0.037) *(n=113)*
MenY	0.044 (0.007-0.097) *(n=114)*	0.036 (0.014-0.068) *(n=111)*
Baseline salivary IgA in ng/mL, median (IQR)		
MenA	42 (26-65) *(n=118)*	38 (26-61) *(n=112)*
MenW	13 (11-17) *(n=120)*	13 (10-16) *(n=115)*
MenY	28 (20-38) *(n=120)*	25 (18-33) *(n=115)*

Abbreviations: ; IgA immunoglobulin A; IQR, interquartile range; MenA, meningococcal serogroup A; MenW, meningococcal serogroup W135; MenY, meningococcal serogroup Y.

Supplementary Table 1B. Characteristics of the study population for meningococcal serogroup C (IgA analyses)

Characteristic		Girls (n=342)	Boys (n=327)
Baseline IgA, median (IQR)			
Serum (µg/mL)			
Overall		0.025 (0.010-0.064) *(n=328)*	0.022 (0.010-0.055) *(n=322)*
10y	14m	0.013 (0.006-0.036) *(n=95)*	0.012 (0.003-0.032) *(n=69)*
12y	3y	0.016 (0.009-0.043) *(n=41)*	0.020 (0.007-0.104) *(n=47)*
12y	14m	0.027 (0.013-0.041) *(n=71)*	0.027 (0.012-0.054) *(n=87)*
15y	6y	0.054 (0.014-0.147) *(n=40)*	0.020 (0.010-0.093) *(n=43)*
15y	3y	0.044 (0.020-0.091) *(n=81)*	0.032 (0.017-0.064) *(n=76)*
Saliva (ng/mL)			
Overall		7.7 (5.0-12.3) *(n=336)*	7.6 (5.0-12.1) *(n=324)*
10y	14m	6.9 (4.0-9.2) *(n=96)*	5.8 (3.6-9.3) *(n=71)*
12y	3y	6.0 (3.0-12.3) *(n=44)*	5.8 (3.5-12.6) *(n=47)*
12y	14m	7.7 (5.4-12.0) *(n=71)*	7.6 (5.4-9.6) *(n=85)*
15y	6y	11.0 (5.8-16.2) *(n=41)*	9.0 (4.2-18.4) *(n=45)*
15y	3y	9.0 (6.3-15.0) *(n=84)*	10.2 (6.9-17.9) *(n=76)*

Abbreviations: IgA immunoglobulin A; IQR, interquartile range; MenC, meningococcal serogroup C; SBA, serum bactericidal antibody.

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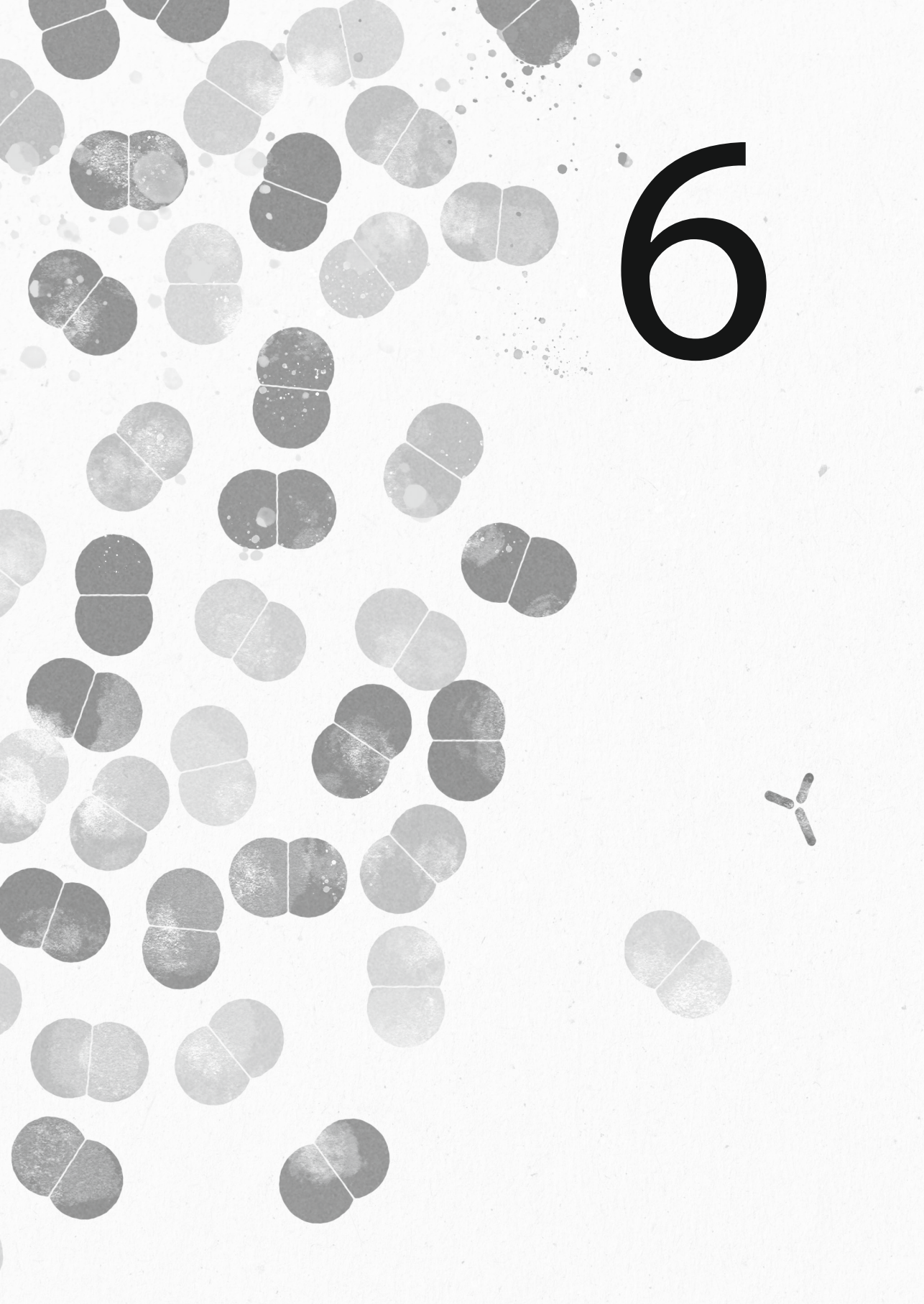

Meningococcal ACWY conjugate vaccine immunogenicity and safety in adolescents with juvenile idiopathic arthritis and inflammatory bowel disease: a prospective observational cohort study

Milou Ohm*[1] & Joeri W van Straalen*[2], Marieke Zijlstra[3], Gerrie de Joode-Smink[2], Anne Jasmijn Sellies[2], Joost F Swart[2,4], Sebastiaan J Vastert[2], Joris M van Montfrans[2,5], Marije Bartels[6], Annet van Royen-Kerkhof[2], Joanne G Wildenbeest[5], Caroline A Lindemans[2,7], Victorien Wolters[3], Roos AW Wennink[8], Joke H de Boer[8], Mirjam J Knol[1], Marloes W Heijstek[9], Elisabeth AM Sanders[1,2], Frans M Verduyn-Lunel[10], Guy AM Berbers[1], Nico M Wulffraat[2], Marc HA Jansen[2]

[1] Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands

[2] Department of Pediatric Immunology and Rheumatology, Wilhelmina Children's Hospital, University Medical Centre Utrecht, Utrecht, the Netherlands

[3] Department of Pediatric Gastroenterology, Wilhelmina Children's Hospital, University Medical Centre Utrecht, Utrecht, the Netherlands

[4] Faculty of Medicine, Utrecht University, Utrecht, the Netherlands

[5] Department of Pediatric Infectious Diseases, Wilhelmina Children's Hospital, University Medical Centre Utrecht, Utrecht, the Netherlands

[6] Department of Pediatric Hematology, Wilhelmina Children's Hospital, University Medical Centre Utrecht, Utrecht, the Netherlands

[7] Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands

[8] Department of Ophthalmology, University Medical Centre Utrecht, Utrecht, the Netherlands

[9] Department of Rheumatology & Clinical Immunology, University Medical Centre Utrecht, Utrecht, the Netherlands

[10] Department of Medical Microbiology, University Medical Centre Utrecht, Utrecht, The Netherlands

* *authors contributed equally*

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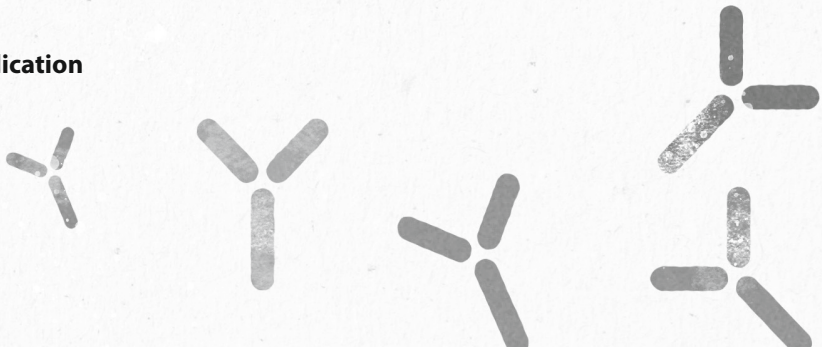

ABSTRACT

Background Immunogenicity to meningococcal serogroup ACWY (MenACWY) conjugate vaccine has not been studied in immunocompromised minors with juvenile idiopathic arthritis (JIA) or inflammatory bowel disease (IBD). We determined immunogenicity of a MenACWY-TT vaccine in adolescents with JIA or IBD and compared results to data from aged-matched healthy controls (HCs).

Methods We performed a prospective observational cohort study in 14-18-year-olds diagnosed with JIA or IBD, who received a MenACWY vaccination during a nationwide catch-up campaign (2018–2019) in the Netherlands. Primary aim was to compare MenACWY polysaccharide-specific serum IgG geometric mean concentrations (GMCs) in patients with HCs and secondary between patients with or without anti-TNF therapy. GMCs were determined before and 3-6, 12, and 24 months postvaccination and compared with data from HCs at baseline and 12 months postvaccination. Serum bactericidal antibody (SBA) titers were determined in a subset of patients at 12 months postvaccination.

Findings We included 226 participants with JIA (66%) or IBD (34%). GMCs were lower for MenA and MenW (GMC ratio 0.24 [0.17-0.34] and 0.16 [0.10-0.26] respectively, $p<0.01$) in patients compared to HCs at 12 months postvaccination. Anti-TNF users had lower MenACWY GMCs postvaccination compared with those without anti-TNF ($p<0.01$). The proportion protected (SBA≥8) for MenW was reduced in anti-TNF users (76% versus 92% in non-anti-TNF and 100% in HCs, $p<0.01$).

Interpretation The MenACWY conjugate vaccine was immunogenic in the vast majority of adolescents with JIA and IBD but seroprotection was lower in patients using anti-TNF agents. Therefore, an extra booster MenACWY vaccination should be considered.

INTRODUCTION

Pediatric patients with autoimmune inflammatory diseases are more susceptible for (a severe course of) infections, which is either caused by the disease itself and/or the use of immunosuppressive or immunomodulating medication[1]. Immunosuppressive/modulatory drugs are fundamental to suppress disease activity, but can lead to a compromised immune system. Juvenile idiopathic arthritis (JIA) is the most common rheumatic condition in children [2] and inflammatory bowel disease (IBD) is an important gastro-intestinal inflammatory disorder in the pediatric population [3]. Even though these disorders vary widely in clinical manifestation, tumor necrosis factor (TNF) plays an important role in the pathophysiology of these diseases and is a key target for therapy for both diseases[4].

Vaccination in immunocompromised patients is crucial to provide better protection against infections. Over the years, trials have proved that the risk of adverse events, such as disease flares, is limited and vaccinations are now advocated for (pediatric) patients with immune disorders [5, 6]. Yet, vaccine immunogenicity was not always found to be as good as in healthy individuals, although data are conflicting [5]. Progress towards better treatment of many immune diseases was made with the introduction of biological Disease Modifying Anti-Rheumatic Drugs (bDMARDs), of which anti-TNF agents are most commonly used. In addition to improvement of therapy, bDMARDS as well as conventional synthetic DMARDS (csDMARDs) may impact the immune system in an unwanted way. Studies show that B-cell depleting therapies and high-dose glucocorticoids hamper the humoral response upon vaccination in children [7]. In addition, recent studies showed that children on TNF inhibitors generally have adequate immune responses upon vaccination but antibody levels were lower and tended to decline more rapidly compared with healthy controls [7, 8].

Previously, meningococcal C (MenC) conjugate vaccination was shown to be immunogenic and safe in patients with JIA [9]. However, data on vaccine-induced antibody responses (including the effect of medication use) and safety in immunocompromised pediatric patients receiving a MenACWY vaccination is lacking. Due to an outbreak of serogroup W invasive meningococcal disease (IMD-W), the MenACWY vaccination was introduced in the national immunization programme (NIP) in the Netherlands for toddlers aged 14 months (replacing MenC conjugate vaccination at 14 months of age) in 2018 and adolescents 14 years of age (newly introduced) in 2020 [10]. Furthermore, a catch-up campaign for all adolescents aged 14-18 years took place in 2018-2019. In order to assess immunogenicity of the MenACWY conjugate vaccine (MenACWY-TT,

Nimenrix©) in adolescents with JIA or IBD, we conducted a prospective observational study in a cohort of adolescent patients 14-18 years of age. Vaccine responses – overall, as well as in relation to medication use – were measured in sera collected pre- and postvaccination, with a follow-up of two years. Safety was evaluated by analyzing the effect of meningococcal vaccination on disease activity and (serious) adverse events in patients.

METHODS

Study design and participants

The MenACWY vaccination was included in the NIP in the Netherlands since 2020 for adolescents aged 14 years, preceded by a nationwide catch-up campaign in 2018–2019 for 14-18 year-olds [10]. An observational cohort study that started at the beginning of the campaign in 2018 was performed in adolescent patients with immune disorders (autoimmune inflammatory rheumatic diseases including JIA, IBD, SLE, MCTD, vasculitis, uveitis, immune deficiencies [cellular and humoral], 22q11 deletion syndrome, sickle cell disease or (functional) asplenia, and patients that underwent stem cell transplantation after bone marrow failure/aplasia). Patients were recruited from the Wilhelmina Children's Hospital of the University Medical Centre Utrecht. For the current study, we asked all JIA and IBD patients who were 14-18 years of age and eligible for vaccination during the campaign to participate. The adolescent patients with other immune disorders as mentioned hereabove will be described elsewhere. All participants received a single dose of MenACWY-TT (Nimenrix®) from the local public health center. Written informed consent was obtained from participants and also their parents/guardians if the participant was under 16 years of age at time of enrollment. The Medical Research Ethics Committee Utrecht decided that the study was exempt from the Medical Research Involving Human Subjects Act (WMO) (local RIB protocol number 18/558/C). Clinical data and collection of blood samples occurred as part of routine follow-up visits with the clinician. Thus, blood samples were collected before, and at 3-6 months, 12 months (+/- 3 months) and 24 months (+/- 3 months) after vaccination. Serology results were compared with healthy control data (15 years of age) at baseline and 12 months postvaccination from a randomized controlled trial that was previously performed and published by National Institute for Public Health and the Environment (RIVM) [11, 12].

Outcome measures

Serology

Meningococcal serogroup A, C, W and Y polysaccharide (PS)-specific serum IgG concentrations were determined by fluorescent bead-based multiplex immunoassay (MIA), as described previously [13]. The lower level of quantitation was set at 0.01 µg/mL. Functional antibodies were determined with the serum bactericidal antibody (SBA) assay in an arbitrarily chosen subset of sera (n=97) at 12 months postvaccination, with a titer ≥8 considered as the protective threshold (internationally-accepted correlate of protection) [14, 15].

Safety

Safety was assessed by determining disease activity and patient's self-reported adverse events (interviewed by the clinician) after vaccination in all participants. Disease activity was assessed at every visit and measured in JIA patients with the clinical Juvenile Arthritis Disease Activity Score including 27 joints (cJADAS-27) with a range from 0 (low activity) to 47 (high activity) [16] and in IBD patients either by the weighted Pediatric Crohn's Disease Activity Index (wPCDAI) [17] or by the Pediatric Ulcerative Colitis Activity Index (PUCAI) [18]. Medication use was noted at each visit. All participants were asked at every visit for (serious) adverse events, which were registered if present.

Statistical analysis

Baseline and follow-up MenACWY-PS specific IgG concentrations were log-transformed prior to all statistical analyses and presented as GMCs with corresponding 95% confidence intervals (CI). GMCs of JIA and IBD patients were compared with data from aged-matched healthy controls (HCs) at baseline and 12 months postvaccination using the ANOVA test [11, 12]. GMCs were compared between anti-TNF users, non-anti-TNF users (i.e. patients who did not use anti-TNF agents, regardless if other biologicals were used) and HCs at baseline and 12 months postvaccination using the ANOVA test. Post-hoc tests were performed using the t-test with Bonferroni correction. GMCs at 3-6 and 24 months postvaccination were compared between anti-TNF and non-anti-TNF users using the t-test. Also, pairwise comparisons of GMCs in anti-TNF and non-anti-TNF users per visit were performed to determine differences between timepoints using the t-test with Bonferroni correction. In order to study the independent effect of anti-TNF use on log-transformed MenACWY IgG concentrations postvaccination in IBD and JIA patients, we performed crude and adjusted linear mixed model analyses [19]. Variables adjusted for in the analyses were sex, disease, age at vaccination, baseline IgG concentration (constant variables), follow-up time and drug therapy (other

than anti-TNF) (time-varying variables). The regression coefficient was exponentiated to obtain (adjusted) GMC ratios and 95% CIs for anti-TNF users versus non-anti-TNF users. For these analyses, we used a random intercept per patient and a random slope for the anti-TNF effect. Missing GMC data were handled by multiple imputation using chained equations [20]. All analyses were run for 20 imputed datasets and estimates were pooled using Rubin's rules. Furthermore, we decided a-priori to perform linear mixed model analyses to study the adjusted effect of follow-up time on MenACWY IgG concentrations postvaccination. In order to assess if this effect was different for anti-TNF and non-anti-TNF users, we added an interaction term between anti-TNF use and follow-up time to the regression models.

We aimed to determine a cut-off for the PS-specific IgG concentrations using antibody data from patients and HCs. The threshold for seroprotectivity was defined as the minimal IgG concentration for which 100% of the SBA titers 12 months postvaccination were protective (SBA≥8) in the healthy controls.

Log-transformed SBA titers for the different serogroups at 12 months postvaccination were compared between anti-TNF users, non-anti-TNF users and healthy controls using the ANOVA test and post-hoc tests were performed using the t-test with Bonferroni correction. Proportions of participants with seroprotective SBA titers (SBA≥8) were compared using Fisher's exact test and post-hoc tests were performed with Bonferroni correction.

An overall difference in disease activity score (cJADAS, PUCAI, wPCDAI) between study visits was tested with the Skillings-Mack test for unbalanced dependent samples [21]. Pairwise comparisons of disease activity scores per visit were performed using the Wilcoxon rank sum test with Bonferroni correction.

A p-value of 0.05 was considered statistically significant for all analyses. All analyses were performed using R version 4.0.3 and the mice and lme4 packages.

RESULTS

Baseline characteristics

Between October 2018 and March 2020, 226 participants (59% female, 134/226) were included (Figure 1) with a median age of 15.7 years (Table 1). Among them, two-thirds of the patients was diagnosed with JIA (150/226, 66%) of which the main subgroups were oligo-and polyarthritis. One-third of the patients had IBD (76/226, 34%), with Crohn's disease as most common subtype. A total of 113 out of 226 patients (50%) used csDMARDs and 109 out of 226 (48.2%) used bDMARDs, mostly anti-TNF agents (89 out of 109).

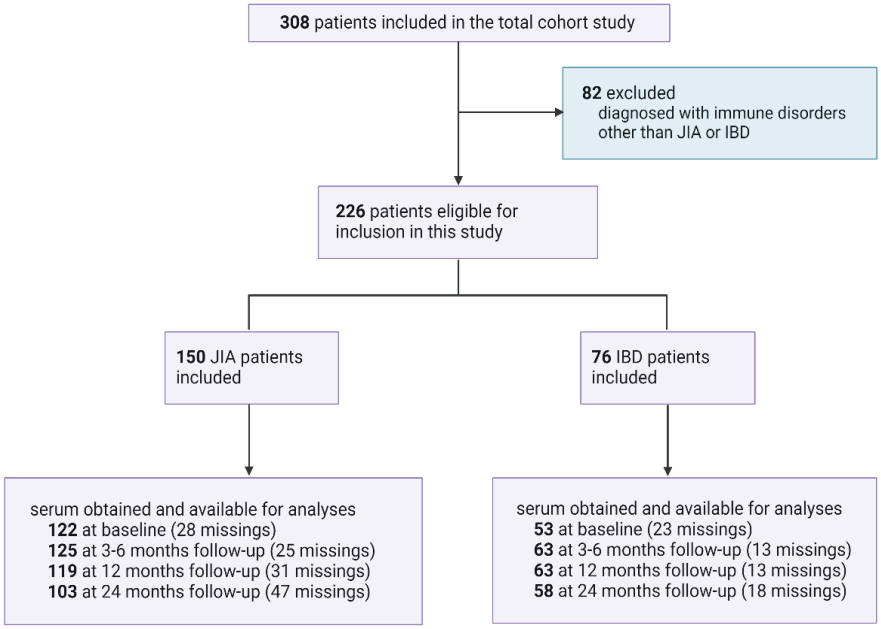

Figure 1. Flow-chart of inclusion. Created with BioRender.com

Table 1. Study participant characteristics at baseline

	Total patient cohort (n = 226)	**JIA (n = 150)**	**IBD (n = 76)**	**Healthy controls (n=75)**
Female sex, n (%)	134 (59.3%)	95 (63.3%)	39 (51.3%)	36 (48%)
Age in years, median (IQR)	15.7 (14.3 – 17.3)	15.6 (14.1 – 17.3)	16.3 (14.9 – 16.7)	15.2 (14.9 – 15.5)
Medication use, n (%) N/A				
No immunosuppressive drugs/NSAIDs	61 (27.0%)	52 (34.7%)	9 (11.8%)	
Systemic corticosteroids	14 (6.2%)	4 (2.7%)	10 (13.2%)	
csDMARDs	113 (50.0%)	67 (44.7%)	46 (60.5%)	
MTX	54 (23.9%)	52 (34.7%)	2 (2.6%)	
AZA	39 (17.3%)	3 (2.0%)	36 (47.4%)	
SSZ	15 (6.6%)	0 (0.0%)	15 (19.7%)	
LEF	7 (3.1%)	7 (4.7%)	0 (0.0%)	
bDMARDs	109 (48.2%)	67 (44.7%)	42 (55.3%)	
Anti-TNF	89 (39.4%)	54 (36.0%)	35 (46.1%)	
Non-anti-TNF bDMARD	20 (8.8%)	13 (8.7%)	7 (9.2%)	
Anti-TNF + csDMARD	55 (24.3%)	36 (24.0%)	19 (25.0%)	
Disease, n (%)				N/A
JIA	150 (66.4%)	150 (100.0%)	N/A	
Persistent oligoarthritis	49 (21. 7 %)	49 (32. 7 %)		
Extended oligoarthritis	16 (7.1%)	16 (10.7%)		
Polyarthritis	47 (20.8%)	47 (31.3%)		
Systemic arthritis	14 (6.2%)	14 (9.3%)		
Enthesitis-related arthritis	11 (4.9%)	11 (7.3%)		
Psoriatic arthritis	8 (3.5%)	8 (5.3%)		
Other JIA	5 (2.2%)	5 (3.3%)		
IBD	76 (33.6%)	N/A	76 (100.0%)	
Crohn's disease	44 (19.5%)		44 (57.9%)	
Ulcerative colitis	21 (9.3%)		21 (27.6%)	
IBD-unclassified	11 (4.9%)		11 (14.5%)	

IQR = interquartile range; NSAIDs = non-steroidal anti-inflammatory drugs; MTX = methotrexate; AZA = azathioprine; SSZ = sulfasalazine; LEF = leflunomide; bDMARDS = biological Disease Modifying Anti-Rheumatic Drugs; csDMARDS = conventional synthetic Disease Modifying Anti-Rheumatic Drugs; JIA = juvenile idiopathic arthritis; IBD = inflammatory bowel disease; N/A = not applicable

Meningococcal polysaccharide-specific IgG concentrations in JIA and IBD patients

GMCs of PS-specific IgG concentrations were below 0.5 µg/mL for all serogroups at baseline in patients (Figure 2 and Supplementary Table 1). Compared with HCs, IgG PS-specific GMCs were significantly lower in patients for serogroup A and W (2.0 [1.6-2.4] and 0.7 [0.5-1.0] respectively versus 8.2 [6.5-10.4] and 4.5 [3.3-6.3] in HCs) at 12 months postvaccination and also at baseline (Supplementary Table 1). GMCs did not differ between patients and HCs for serogroup C and Y at 12 months postvaccination. Three months after vaccination, GMCs significantly increased compared to baseline (Supplementary Table 2) and the GMC for MenC was significantly higher than for MenAWY (Figure 2). MenA and MenC antibodies waned over time between 3-6 months and 12 months postvaccination ($p<0.01$), and between 3-6 months and 24 months postvaccination for all serogroups ($p<0.01$) (Supplementary Table 2).

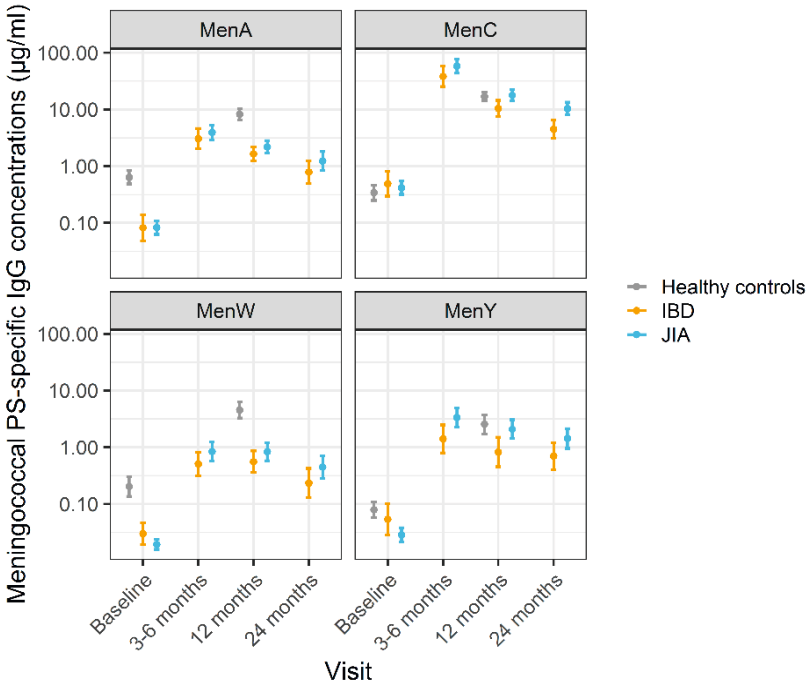

Figure 2. Meningococcal serogroup A, C, W and Y polysaccharide-specific serum IgG concentrations in JIA patients, IBD patients and healthy controls at all timepoints. Dots indicate geometric mean concentrations with 95% confidence intervals. JIA = juvenile idiopathic arthritis; IBD = inflammatory bowel disease; PS = polysaccharide

Effect of anti-TNF agents on PS-specific IgG concentrations in JIA and IBD patients

Within the patient cohort, non-anti-TNF users were more often female (66%) while anti-TNF users were slightly more often males (52%) using anti-TNF (Supplementary Table 3). No difference in systemic corticosteroid use was found between anti-TNF users and non-anti-TNF users. Among non-anti-TNF users, 42% used sDMARDS, 22% used methotrexate and 15% used bDMARDS other than anti-TNF. For all serogroups, significant differences in PS-specific IgG GMCs between anti-TNF users and non-anti-TNF users were already present 3-6 months postvaccination (Figure 3, Supplementary Table 4) and these differences persisted until 24 months postvaccination. Both the crude and adjusted effect of anti-TNF therapy at baseline on PS-specific IgG concentrations were statistically significant for all serogroups in the linear mixed model (Table 2, Supplementary Table 5). The GMC ratio between anti-TNF users and non-anti-TNF users was lowest for serogroup Y (0.19 [0.10-0.34]) and highest for serogroup A (0.50 [0.33-0.76]) in the adjusted analysis (Table 2), but also significant for serogroup C (0.47 [0.32-0.70]) and serogroup W (0.23 [0.14-0.39]). A difference in GMC between 12 and 24 months postvaccination was observed for serogroup C and W ($p<0.01$) but not for A and Y in anti-TNF users. In non-anti-TNF users, significant differences in GMCs between 12 and 24 months were found for serogroup A and C (Supplementary Table 6).

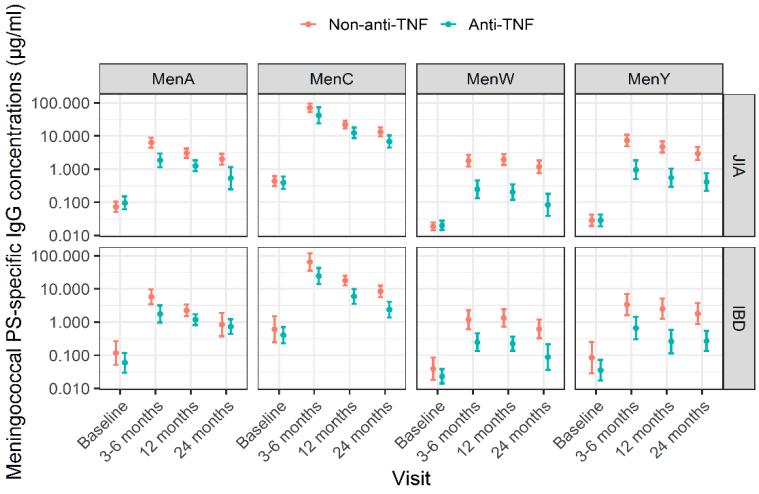

Figure 3. Meningococcal serogroup A, C, W and Y polysaccharide-specific serum IgG concentrations in anti-TNF users and non-anti-TNF users per disease cohort (in JIA and IBD patients) at baseline and during follow-up. Dots indicate geometric mean concentrations with 95% confidence intervals. JIA = juvenile idiopathic arthritis; IBD = inflammatory bowel disease; PS = polysaccharide

Table 2. Linear mixed model analyses for the independent effect of anti-TNF use at baseline on log-transformed meningococcal IgG concentrations at all postvaccination timepoints for JIA and IBD patients

Serogroup	Analysis	GMC ratio for anti-TNF users vs. non-anti-TNF users (95% CI)
MenA	Crude	0.44 (0.31 - 0.63)*
	Adjusted[1]	0.50 (0.33 - 0.76)*
MenC	Crude	0.50 (0.35 - 0.71)*
	Adjusted[1]	0.47 (0.32 - 0.70)*
MenW	Crude	0.17 (0.11 - 0.28)*
	Adjusted[1]	0.23 (0.14 - 0.39)*
MenY	Crude	0.14 (0.08 - 0.24)*
	Adjusted[1]	0.19 (0.10 - 0.34)*

*statistically significant effect

[1]adjusted for sex, disease, age at vaccination, baseline IgG concentration (constant variables), follow-up time and immunosuppressive drug therapy other than anti-TNF (time-varying variables)

Missing values were handled by multiple imputation. GMC = geometric mean concentration

Functional antibodies at 12 months postvaccination

Serum samples from a random subset of n=97 patients (of which 65 diagnosed with JIA and 32 diagnosed with IBD) collected at 12 months postvaccination were tested in the SBA assay. We compared three different groups: anti-TNF users, non-anti-TNF users and HCs. The seroprotection rates (proportion with SBA titer ≥8) between patients using anti-TNF, patients not using anti-TNF and HCs were significantly different for MenW (76%, 92%, and 100% respectively, $p<0.01$), but not for MenACY (Table 3). Furthermore, SBA GMTs at 12 months postvaccination were significantly lower ($p<0.05$) for serogroup C and W in the anti-TNF group in comparison with the non-anti-TNF group (Supplementary Table 7). There were no significant differences in GMTs between the anti-TNF and non-anti-TNF group for serogroup A, and Y. However, significant differences between GMTs were found for all serogroups when anti-TNF users were compared with HCs (Supplementary Table 6). The lowest SBA GMT was observed for serogroup W, with a GMT of 188 [80-440] in the anti-TNF group, compared with 533 [304-934] in the non-anti-TNF group and 1546 [1257-1903] in HCs. We did not find a difference between boys and girls in the protected proportion of JIA and IBD patients (Supplementary Table 8). Because functional antibody titers did not correlate with PS-specific IgG concentrations except for serogroup C ($r=0.88$, $p<0.01$), a cut-off for IgG seroprotection could not be determined (Supplementary Figure 1 and Supplementary Table 9); not all children with a low SBA titer also showed a low IgG concentration, some had IgG concentrations above 0.5 or even 1.0 µg/mL (Supplementary Figure 1).

Table 3. Frequency (%) of seroprotective SBA titres (≥8) 12 months postvaccination in JIA and IBD patients with and without anti-TNF use at baseline and in healthy 15 year-old controls (HC).

Serogroup	Non-anti-TNF (n = 52)	Anti-TNF (n = 45)	HC (n = 75)	*P*-value			
				Overall difference	Non-anti-TNF vs. anti-TNF[1]	Non-anti-TNF vs. HC[1]	Anti-TNF vs. HC[1]
MenA	50 (96%)	41 (91%)	74 (99%)	0.11	1.00	1.00	0.20
MenC	49 (94%)	44 (98%)	75 (100%)	0.06	1.00	0.20	1.00
MenW	48 (92%)	34 (76%)	74 (100%)[2]	<0.01*	0.08	0.08	<0.01*
MenY	49 (96%)[2]	43 (96%)	73 (97%)	0.88	1.00	1.00	1.00

*$P < 0.05$

[1]*P*-values were adjusted for multiple testing with Bonferroni correction

[2]one missing observation

Safety: disease activity and adverse events

No severe adverse events were reported during the study. Three patients reported an event of special interest during routine care at 3 months follow-up, which included worsening of alopecia areata, low serum adalimumab level, and sinusitis. All events were transient. No significant overall difference was observed in disease activity scores (wPCDAI, PUCAI and cJADAS) during follow-up (Figure 4) and at 3 months postvaccination compared to baseline (Supplementary Table 10).

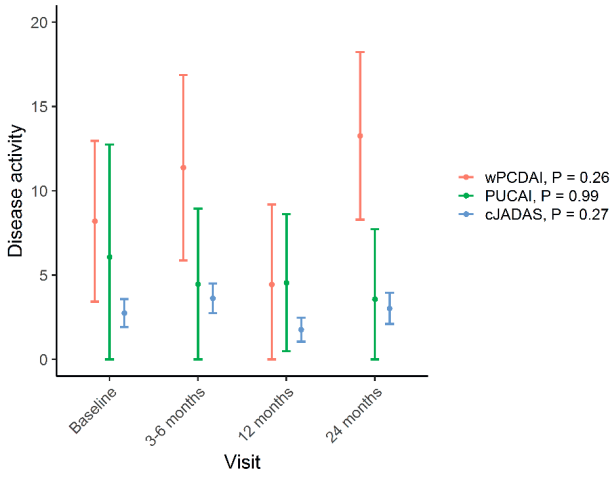

Figure 4. Disease activity as measured with the wPCDAI, PUCAI or cJADAS at all timepoints. Dots indicate mean disease activity scores with 95% confidence intervals. P-values reflect an overall difference between study visits. cJADAS = clinical Juvenile Arthritis Disease Activity Score; wPCDAI = weighted Pediatric Crohn's Disease Activity; PUCAI = Pediatric Ulcerative Colitis Activity Index (PUCAI)

DISCUSSION

In this study, we found that a single dose of meningococcal ACWY conjugate vaccine is immunogenic and in general elicited seroprotective antibody titers in adolescents diagnosed with JIA or IBD. However, the vaccine was less immunogenic in patients using anti-TNF agents compared with patients not using anti-TNF agents and compared with healthy controls. More specifically, one fourth of the patients on anti-TNF did not have a protective functional antibody titer against serogroup W 12 months after vaccination. No severe advents or increase in disease activity was detected after vaccination.

The few studies reporting on the vaccine response in pediatric patients treated with bDMARDs are contradictory and not always in line with our results. In a recently published systematic literature review that assessed all available data on vaccines except for the COVID-19 vaccines, pediatric patients with autoimmune inflammatory rheumatic diseases did not have lower seroprotection rates when bDMARDS were used except for B-cell depleting therapies [7]. The only available reports on meningococcal vaccines found that MenC vaccination was safe and immunogenic in JIA patients [9], although an accelerated decline of antibodies was observed when biologicals were used [22]. Current guidelines recommend regular vaccinations according to the NIP[7], but recommendations on determination of seroprotection levels postvaccination, and consequently a booster vaccination in case of low antibody levels, are lacking.

TNF inhibitors suppress the response to TNF, a cytokine involved in immune and inflammatory responses such as proliferation and activation of T-cells, B-cells, macrophages, dendritic cells and NK cells [23]. Although the pathogenesis of some immune-mediated inflammatory diseases – including JIA and IBD - remains incompletely understood, an excessive production of proinflammatory cytokines including TNFα is involved and plays a crucial role in treatment. The immune response to a conjugate vaccine includes the activation of B- and T-cells that results in the production of antibodies and induction of a cellular memory response. TNF promotes the activation and proliferation of T cells, both naïve and effector, and can thereby provide help to B-cells for antibody production. Anti-TNF may alter the T-cell dependent B-cell response, which is especially important in the polysaccharide-specific B-cell response that is induced by conjugate vaccines [24]. Furthermore, TNF induces dendritic cell maturation, which promotes an efficient antigen presentation [25]. CD40 and the CD40 ligand, which are important proteins in the carrier-peptide-specific T cell response, have been reported to be down-regulated by anti-TNF agents in patients with Crohn's

disease [24, 26]. Future research should investigate how the recall response to an extra booster vaccination (while being treated with anti-TNF) is influenced.

In this study, most patients were primed with a MenC vaccination (vaccine uptake was around 95% in 2006-2008 [27]) at the age of 14 months. The response to a new antigen differs from a recall response and secondary responses are less likely to be impaired by immunosuppressive therapy [28]. We indeed observed a higher GMC in MenC compared to the other serogroups in both patients with or without anti-TNF treatment (Supplementary Table 11). This probably predicts a promising booster response for the other 3 meningococcal serogroups as well - even when anti-TNF agents are used - which underlines the importance of a booster vaccination, especially in patients who did not respond (fully) to the primary vaccination. Since the MenC vaccination was replaced for a MenACWY vaccination in the Dutch NIP, future patients (the first children primed at the age of 14 months with MenACWY vaccination in 2019 will receive a booster at 14 years around the year 2032) will probably respond to all serogroups as a recall. Thus, an additional booster vaccination for these patients might become unnecessary by the time MenACWY-primed toddlers receive the MenACWY booster vaccination as an adolescent.

One year postvaccination, one quarter of the patients using anti-TNF was not protected for MenW in this study and we expect that this proportion will further increase over time. We found that vaccine-induced PS-specific serum IgG concentrations were unreliable as cut-off for seroprotection as measured by SBA, as not all children with a low SBA titer also showed a low IgG concentration, some even had IgG concentrations above 0.5 or even 1.0 µg/mL. The functionality of the all antibodies in addition to serum components as complement proteins (as reflected by the SBA) involves not only PS-specific IgG, but also other antibodies not restricted to the capsule and also for example IgM. Children that have a low SBA titer despite adequate PS-specific IgG concentrations may therefore actually benefit from an extra booster vaccination. Thus, PS-specific IgG concentrations were unreliable to use as a cut-off, which hampers individual-based advice on a booster vaccination for each patient by physicians. The SBA assay is however an expensive and time-consuming assay, and only validated for research purposes. Since the antibody decay, rather than hyporesponsiveness to the initial vaccination, might play a role in the reduced protection induced by vaccination [22], a booster vaccination should be considered. Usually, a 2-dose schedule or a vaccination 3-5 years after the primary vaccination is advised in risk groups. A 2-dose schedule may induce a good initial vaccine response, but does not necessarily lead to a longer duration of protection [29]. A booster could provide this, but earlier boosting

(earlier than after 3-5 years) is required to provide protection for at least one-fourth of the patients that would otherwise be unprotected during the period in life that an individual has high risk of contracting the meningococcal bacterium. For the clinical practice, therefore, we propose that an extra MenACWY vaccination should be considered for all adolescents treated with anti-TNF, regardless of IgG concentration, one year after the regular vaccination.

While safety has not been investigated before in immunocompromised adolescents receiving a MenACWY vaccination, for MenC vaccination safety was proved to be assured and no adverse events were reported [9]. We did not find altered disease activity three months after MenACWY vaccination in JIA and IBD patients and no safety issues were reported in patients using immunosuppressive/modulating agents. This is in line with what was found for other vaccines [30].

Our study comes with limitations, especially because we performed an observational cohort study. Serum sampling depended on routine visits with the clinician and the COVID-19 pandemic has led to dropouts during follow-up. Furthermore, the age of vaccination in this study was 14-18 years, while currently in the NIP adolescents receive MenACWY vaccination at 14 years. Therefore, we might have overestimated the vaccine response since this may increase with age [12].

Strengths of the study were that we assessed functional antibody activity (SBA assay) – in addition to IgG concentrations – to actually assess seroprotection rates. We were able to take into account medical data including disease activity and medication use such as anti-TNF agents. We prospectively followed-up on patients for 24 months and could therefore optimize the recommendations for a possible booster vaccination. Furthermore, we investigated both differences between anti-TNF users and non-anti-TNF users as well as the difference between healthy adolescents and patients (with or without medication use) by including healthy age-matched control data. In addition, we performed analyses to adjust for dependent measurements within patients over time and factors that could have led to confounding, which is a frequent problem in observational studies. We encourage that our results are validated in another prospective cohort.

In conclusion, vaccination of immunocompromised adolescents with a MenACWY conjugate vaccine was immunogenic, but patients using anti-TNF agents showed lower antibody concentrations for all serogroups and even reduced seroprotection rates for MenW. An extra booster vaccination in those adolescents should be consid-

ered, which we would now advise one year after the regular adolescent vaccination at the age of 14 years. Future research should evaluate the effect and optimal timing of a booster vaccination.

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Supplementary Table 1. Geometric mean concentrations (GMCs) and 95% confidence intervals of meningococcal polysaccharide-specific serogroup A, C, W and Y (MenACWY) IgG concentrations (µg/ml) during follow-up in the patient cohort (juvenile idiopathic arthritis (JIA) and inflammatory bowel disease (IBD) patients) and in healthy controls (HC).

Months	Serogroup	JIA + IBD	HC	P-value	GMC ratio (95% CI)
0		n = 175	n = 75		
	MenA	0.1 (0.1 – 0.1)	0.6 (0.5 – 0.8)[2]	<0.01*	**0.13 (0.08 – 0.20)**
	MenC	0.4 (0.3 – 0.6)	0.3 (0.3 – 0.5)	0.19	1.29 (0.85 – 1.95)
	MenW	0.0 (0.0 – 0.0)	0.2 (0.1 – 0.3)	<0.01*	**0.11 (0.07 – 0.16)**
	MenY	0.0 (0.0 – 0.0)	0.1 (0.1 – 0.1)	<0.01*	**0.44 (0.27 – 0.70)**
3-6		n = 188	n = 0		
	MenA	3.6 (2.8 – 4.6)	-		
	MenC	50.5 (40.0 – 63.8)	-		
	MenW	0.7 (0.5 – 1.0)	-		
	MenY	2.5 (1.8 – 3.5)	-		
12		n = 182	n = 75		
	MenA	2.0 (1.6 – 2.4)	8.2 (6.5 – 10.4)	<0.01*	**0.24 (0.17 – 0.34)**
	MenC	14.8 (12.3 – 17.8)	16.9 (14.0 – 20.4)	0.32	0.88 (0.64 – 1.19)
	MenW	0.7 (0.5 – 1.0)	4.5 (3.3 – 6.3)	<0.01*	**0.16 (0.10 – 0.26)**
	MenY	1.5 (1.1 – 2.1)	2.5 (1.7 – 3.8)	0.05	0.60 (0.34 – 1.05)
24		n = 161	n = 0		
	MenA	1.0 (0.8 – 1.4)	-		
	MenC	7.7 (6.2 – 9.5)	-		
	MenW	0.4 (0.3 – 0.5)	-		
	MenY	1.1 (0.8 – 1.5)	-		

P-values to compare GMCs were determined with a t-test on log-transformed data. Significant GMC ratios are outlined in bold. *$P < 0.05$

Supplementary Table 2. *P*-values for pairwise comparisons of meningococcal serogroup A, C, W and Y (MenACWY) polysaccharide-specific IgG concentrations at different study visits for juvenile idiopathic arthritis (JIA) and inflammatory bowel disease (IBD) patients.

Serogroup	Time-point	Baseline	3-6 months	12 months
MenA	3-6 months	<0.01*	-	-
	12 months	<0.01*	<0.01*	-
	24 months	<0.01*	<0.01*	<0.01*
MenC	3-6 months	<0.01*	-	-
	12 months	<0.01*	<0.01*	-
	24 months	<0.01*	<0.01*	<0.01*
MenW	3-6 months	<0.01*	-	-
	12 months	<0.01*	1.00	-
	24 months	<0.01*	<0.01*	<0.01*
MenY	3-6 months	<0.01*	-	-
	12 months	<0.01*	0.13	-
	24 months	<0.01*	<0.01*	1.00

*$P < 0.05$

Supplementary Table 3. Study participant baseline characteristics in anti-TNF users and non-anti-TNF users

	Non-anti-TNF (n = 137)	**Anti-TNF** (n = 89)
Female sex, n (%)	91 (66.4%)	43 (48.3%)
Age in years, median (IQR)	15.9 (14.4 - 17.3)	15.7 (13.9 - 16.8)
Medication use, n (%)		
No immunosuppressive drugs/NSAIDs	61 (44.5%)	0 (0.0%)
Systemic corticosteroids	7 (5.1%)	7 (7.9%)
csDMARDs	58 (42.3%)	55 (61.8%)
MTX	30 (21.9%)	24 (27.0%)
AZA	20 (14.6%)	19 (21.3%)
SSZ	9 (6.6%)	6 (6.7%)
LEF	1 (0.7%)	6 (6.7%)
bDMARDs	20 (14.6%)	89 (100.0%)
Anti-TNF	0 (0.0%)	89 (100.0%)
Non-anti-TNF bDMARD	20 (14.6%)	0 (0.0%)
Anti-TNF + sDMARD	0 (0.0%)	55 (61.8%)
Disease, n (%)		
JIA	96 (70.1%)	54 (60.7%)
Persistent oligoarthritis	32 (23.4%)	17 (19.1%)
Extended oligoarthritis	7 (5.1%)	9 (10.1%)
Polyarthritis	28 (20.4%)	19 (21.3%)
Systemic arthritis	14 (10.2%)	0 (0.0%)
Enthesitis-related arthritis	7 (5.1%)	4 (4.5%)
Psoriatic arthritis	5 (3.6%)	3 (3.4%)
Other JIA	3 (2.2%)	2 (2.2%)
IBD	41 (29.9%)	35 (39.3%)
Crohn's disease	22 (16.1%)	22 (24.7%)
Ulcerative colitis	12 (8.8%)	9 (10.1%)
IBD-unclassified	7 (5.1%)	4 (4.5%)

IQR = interquartile range; NSAIDs = non-steroidal anti-inflammatory drugs; MTX = methotrexate; AZA = azathioprine; SSZ = sulfasalazine; LEF = leflunomide; bDMARDS = biological Disease Modifying Anti-Rheumatic Drugs; csDMARDS = conventional synthetic Disease Modifying Anti-Rheumatic Drugs; JIA = juvenile idiopathic arthritis; IBD = inflammatory bowel disease

Supplementary Table 4. Geometric mean concentrations (95% CIs) of meningococcal serogroup A, C, W and Y (MenACWY) polysaccharide-specific IgG concentrations (µg/ml) for juvenile idiopathic arthritis (JIA) and inflammatory bowel disease (IBD) patients with and without anti-TNF use at baseline.

Time-point	Serogroup	Non-anti-TNF	Anti-TNF	HC	*P*-value			
					Overall	Non-anti-TNF vs. anti-TNF[1]	Non-anti-TNF vs. HC[1]	Anti-TNF vs. HC[1]
Baseline		n = 95	n = 80	n = 75				
	MenA	0.1 (0.1 – 0.1)	0.1 (0.1 – 0.1)	0.6 (0.5 – 0.8)[2]	<0.01*	1.00	<0.01*	<0.01*
	MenC	0.5 (0.3 – 0.7)	0.4 (0.3 – 0.6)	0.3 (0.2 – 0.5)	0.35	1.00	0.45	1.00
	MenW	0.0 (0.0 – 0.0)	0.0 (0.0 – 0.0)	0.2 (0.1 – 0.3)	<0.01*	1.00	<0.01*	<0.01*
	MenY	0.0 (0.0 – 0.1)	0.0 (0.0 – 0.0)	0.1 (0.1 – 0.1)	<0.01*	1.00	0.02*	<0.01*
3-6 months		n = 106	n = 82	n = 0				
	MenA	6.2 (4.6 – 8.2)	1.8 (1.3 – 2.6)	-		<0.01*		
	MenC	69.2 (52.8 – 90.7)	33.6 (22.6 – 49.9)	-		<0.01*		
	MenW	1.6 (1.1 – 2.3)	0.2 (0.2 – 0.4)	-		<0.01*		
	MenY	5.9 (4.2 – 8.5)	0.8 (0.5 – 1.3)	-		<0.01*		
12 months		n = 106	n = 76	n = 75				
	MenA	2.8 (2.2 – 3.6)	1.2 (0.9 – 1.6)	8.2 (6.5 – 10.4)	<0.01*	<0.01*	<0.01*	<0.01*
	MenC	20.8 (16.9 – 25.6)	9.2 (6.8 – 12.5)	16.9 (14.0 – 20.4)	<0.01*	<0.01*	0.64	<0.01*
	MenW	1.7 (1.3 – 2.4)	0.2 (0.1 – 0.3)	4.5 (3.3 – 6.3)	<0.01*	<0.01*	<0.01*	<0.01*
	MenY	3.9 (2.8 – 5.5)	0.4 (0.2 – 0.7)	2.5 (1.7 – 3.8)	<0.01*	<0.01*	0.41	<0.01*
24 months		n = 94	n = 67	n = 0				
	MenA	1.5 (1.1 – 2.2)[2]	0.6 (0.4 – 1.0)	-		<0.01*		
	MenC	11.6 (9.0 – 14.8)[2]	4.3 (3.0 – 6.1)	-		<0.01*		
	MenW	1.0 (0.7 – 1.4)	0.1 (0.0 – 0.2)	-		<0.01*		
	MenY	2.5 (1.7 – 3.7)	0.3 (0.2 – 0.5)	-		<0.01*		

*$P < 0.05$

[1]*P*-values at baseline and 12 months were adjusted for multiple testing with Bonferroni correction

[2]one missing observation

HC = healthy controls

Supplementary Table 5. Linear mixed model analyses in juvenile idiopathic arthritis (JIA) and inflammatory bowel disease (IBD) patients for a difference in adjusted effect of follow-up time on log-transformed meningococcal serogroup A, C, W and Y (MenACWY) polysaccharide-specific IgG concentrations postvaccination between anti-TNF and non-anti-TNF use at baseline.

Serogroup	Variable	β (95% CI) [1]
MenA	Follow-up time (years)	-0.77 (-0.96 – -0.59)*
	Follow-up time (years)*anti-TNF use	0.14 (-0.17 – 0.45)
MenC	Follow-up time (years)	-1.14 (-1.30 – -0.98)*
	Follow-up time (years)*anti-TNF use	-0.07 (-0.34 – 0.20)
MenW	Follow-up time (years)	-0.45 (-0.68 – -0.22)*
	Follow-up time (years)*anti-TNF use	-0.13 (-0.58 – 0.31)
MenY	Follow-up time (years)	-0.58 (-0.78 – -0.38)*
	Follow-up time (years)*anti-TNF use	0.04 (-0.32 – 0.40)

*statistically significant effect

[1]adjusted for anti-TNF use at baseline, sex, disease, age at vaccination, baseline IgG concentration (constant variables) and drug therapy (time-varying variable)

JIA = juvenile idiopathic arthritis; IBD = inflammatory bowel disease

Supplementary Table 6. *P*-values for pairwise comparisons of meningococcal serogroup A, C, W and Y (MenACWY) polysaccharide-specific IgG concentrations at different study visits for juvenile idiopathic arthritis (JIA) and inflammatory bowel disease (IBD) patients with and without anti-TNF use at baseline.

Serogroup	Anti-TNF use at baseline	Time-point	Baseline	3-6 months	12 months
MenA	No	3-6 months	<0.01*	-	-
		12 months	<0.01*	<0.01*	-
		24 months	<0.01*	<0.01*	0.04*
	Yes	3-6 months	<0.01*	-	-
		12 months	<0.01*	0.84	-
		24 months	<0.01*	<0.01*	0.07
MenC	No	3-6 months	<0.01*	-	-
		12 months	<0.01*	<0.01*	-
		24 months	<0.01*	<0.01*	0.01*
	Yes	3-6 months	<0.01*	-	-
		12 months	<0.01*	<0.01*	-
		24 months	<0.01*	<0.01*	0.02*
MenW	No	3-6 months	<0.01*	-	-
		12 months	<0.01*	1.00	-
		24 months	<0.01*	0.20	0.08
	Yes	3-6 months	<0.01*	-	-
		12 months	<0.01*	1.00	-
		24 months	<0.01*	<0.01*	0.02*
MenY	No	3-6 months	<0.01*	-	-
		12 months	<0.01*	0.57	-
		24 months	<0.01*	<0.01*	0.64
	Yes	3-6 months	<0.01*	-	-
		12 months	<0.01*	0.16	-
		24 months	<0.01*	0.05*	1.00

*$P < 0.05$

Supplementary Table 7. Geometric mean titres (95% CI) of meningococcal serogroup A, C, W and Y (MenACWY) serum bactericidal antibody (SBA) results per serogroup 12 months postvaccination for juvenile idiopathic arthritis (JIA) and inflammatory bowel disease (IBD) patients with and without anti-TNF use at baseline and healthy aged-matched controls (HC).

Serogroup	**No anti-TNF** (n = 52)	**Anti-TNF** (n = 45)	**HC** (n = 75)	***P*-value**			
				Overall	No anti-TNF vs. anti-TNF[1]	No anti-TNF vs. HC[1]	Anti-TNF vs. HC[1]
MenA	625 (385 – 1015)	413 (222 – 768)	875 (687 – 1115)	0.04*	0.60	0.72	0.04*
MenC	1611 (911 – 2850)	671 (406 – 1111)	2964 (2340 – 3755)	<0.01*	0.02*	0.10	<0.01*
MenW	533 (304 – 934)	188 (80 – 440)	1546 (1257 – 1903)[2]	<0.01*	0.03*	<0.01*	<0.01*
MenY	983 (611 – 1581)[2]	708 (442 – 1133)	1611 (1154 – 2247)	0.02*	0.91	0.25	0.02*

*$P < 0.05$

[1]*P*-values were adjusted for multiple testing with Bonferroni correction

[2]one missing observation

Supplementary Table 8. Frequency (%) of seroprotective meningococcal serogroup A, C, W and Y (MenACWY) serum bactericidal antibody (SBA) titers (≥8) 12 months postvaccination in male and female juvenile idiopathic arthritis (JIA) and inflammatory bowel disease (IBD) patients.

Serogroup	**Girls** (n = 60)	**Boys** (n = 37)	***P*-value**
MenA	55 (91.7%)	36 (97.3%)	0.40
MenC	56 (93.3%)	37 (100.0%)	0.29
MenW	51 (85.0%)	31 (83.8%)	1.00
MenY	57[1] (96.6%)	35 (94.6%)	0.64

[1]one missing observation

Supplementary Table 9. Correlation between polysaccharide-specific serum IgG concentrations and serum bactericidal antibody titers determined with Spearman's correlation at 12 months postvaccination.

Serogroup	***r***	***P*-value**
MenA	0.05	0.60
MenC	0.88	<0.01
MenW	0.12	0.24
MenY	0.19	0.07

Supplementary Table 10. Bonferroni-adjusted *P*-values for pairwise comparisons of disease activity scores at different study visits for juvenile idiopathic arthritis (JIA) and inflammatory bowel disease (IBD) patients.

Score	Time-point	Baseline	3 months	12 months
cJADAS	Baseline (n = 98)			
	3-6 months (n = 90)	1.00	-	-
	12 months (n = 84)	0.39	1.00	-
	24 months (n = 66)	0.71	0.13	0.01*
wPCDAI	Baseline (n = 18)			
	3-6 months (n = 23)	0.95	-	-
	12 months (n = 20)	1.00	1.00	-
	24 months (n = 9)	1.00	0.31	1.00
PUCAI	Baseline (n = 14)			
	3-6 months (n = 14)	1.00	-	-
	12 months (n = 13)	1.00	1.00	-
	24 months (n = 11)	1.00	1.00	1.00

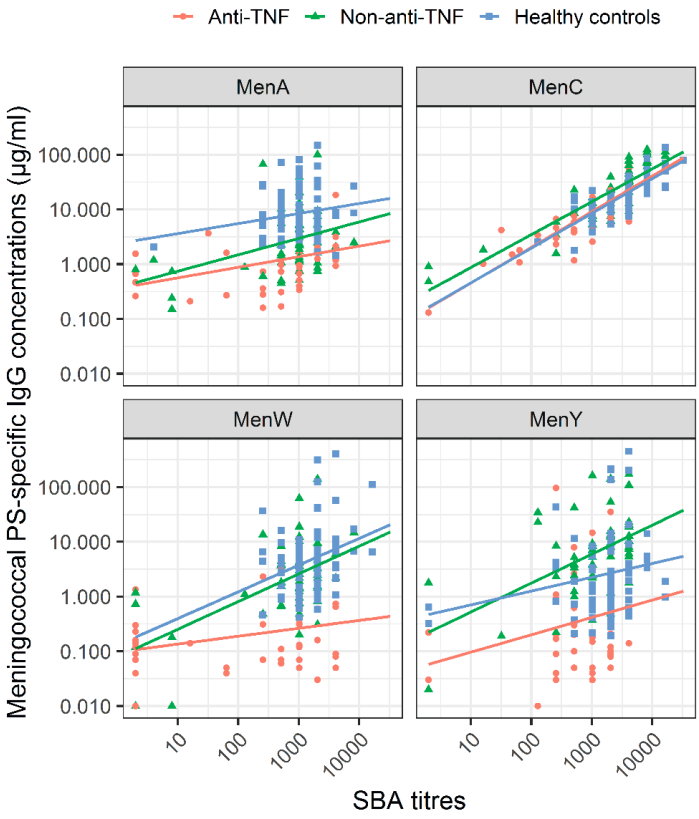
*$P < 0.05$

Supplementary Table 11. Geometric mean concentrations and 95% confidence intervals of meningococcal serogroup A, C, W and Y (MenACWY) polysaccharide-specific IgG concentrations (µg/ml) in juvenile idiopathic arthritis (JIA) and inflammatory bowel disease (IBD) patients during follow-up.

Timepoint	Anti-TNF use at baseline	MenA	MenC	MenW	MenY	*P*-value
0 months	No (n = 95)	0.08 (0.06 – 0.12)	0.48 (0.34 – 0.67)	0.02 (0.02 – 0.03)	0.04 (0.03 – 0.06)	<0.01*
	Yes (n = 80)	0.08 (0.06 – 0.12)	0.40 (0.28 – 0.56)	0.02 (0.02 – 0.03)	0.03 (0.02 – 0.04)	<0.01*
3-6 months	No (n = 106)	6.2 (4.6 – 8.2)	69.2 (52.8 – 90.7)	1.6 (1.1 – 2.3)	5.9 (4.2 – 8.5)	<0.01*
	Yes (n = 82)	1.8 (1.3 – 2.6)	33.6 (22.6 – 49.9)	0.2 (0.2 – 0.4)	0.8 (0.5 – 1.3)	<0.01*
12 months	No (n = 106)	2.8 (2.2 – 3.6)	20.8 (16.9 – 25.6)	1.7 (1.3 – 2.4)	3.9 (2.8 – 5.5)	<0.01*
	Yes (n = 76)	1.2 (0.9 – 1.6)	9.2 (6.8 – 12.5)	0.2 (0.1 – 0.3)	0.4 (0.2 – 0.7)	<0.01*
24 months	No (n = 94)	1.5[1] (1.1 – 2.2)	11.6[1] (9.0 – 14.8)	1.0 (0.7 – 1.4)	2.5 (1.7 – 3.7)	<0.01*
	Yes (n = 67)	0.6 (0.4 – 1.0)	4.3 (3.0 – 6.1)	0.1 (0.0 – 0.2)	0.3 (0.2 – 0.5)	<0.01*

*statistically significant

[1]one missing observation



Supplementary Figure 1. Plots of meningococcal serogroup A, C, W and Y polysaccharide-specific serum IgG concentrations versus serum bactericidal antibody (SBA) titers for participants with available data 12 months postvaccination. Coloured lines indicate linear trends.

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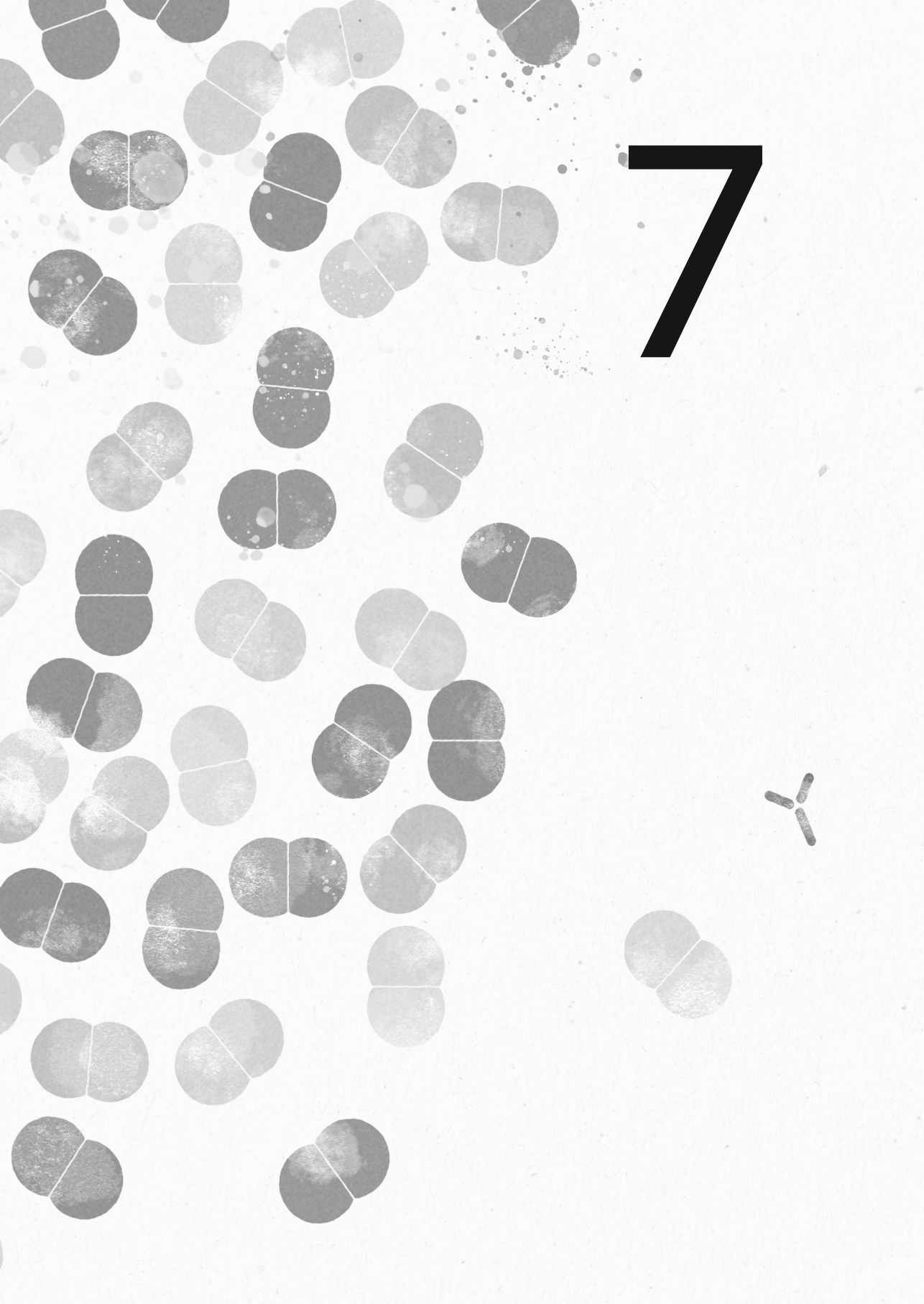

Meningococcal ACWY conjugate vaccine immunogenicity in adolescents with primary or secondary immune deficiencies, a prospective observational cohort study

Milou Ohm*[1] & Joeri W van Straalen*[2], Gerrie de Joode-Smink[2], Joris van Montfrans[2,4], Marije Bartels[3], Joanne G van Wildenbeest[4], Caroline A Lindemans[2,5], Roos AW Wennink[6], Joke H de Boer[6], Elisabeth AM Sanders[1], Frans M Verduyn-Lunel[7], Guy AM Berbers[1], Nico M Wulffraat[2], Marc HA Jansen[2]

[1] Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands

[2] Department of Pediatric Immunology and Rheumatology, Wilhelmina Children's Hospital, University Medical Centre Utrecht, Utrecht, the Netherlands

[3] Department of Pediatric Hematology, Wilhelmina Children's Hospital, University Medical Centre Utrecht, Utrecht, the Netherlands

[4] Department of Pediatric Infectious Diseases, Wilhelmina Children's Hospital, University Medical Centre Utrecht, Utrecht, the Netherlands

[5] Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands

[6] Department of Ophthalmology, University Medical Centre Utrecht, Utrecht, the Netherlands

[7] Department of Medical Microbiology, University Medical Centre Utrecht, Utrecht, The Netherlands

* *authors contributed equally*

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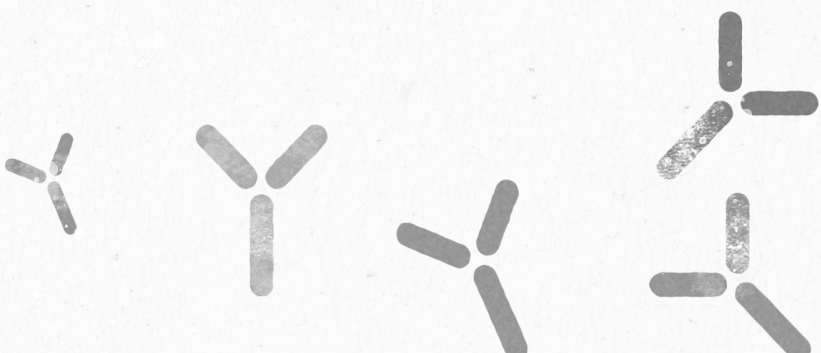

ABSTRACT

Background Immunization with meningococcal ACWY conjugate vaccine induces protective antibodies against invasive meningococcal disease (IMD) caused by serogroups A, C, W and Y. We studied MenACWY-TT vaccine immunogenicity in adolescents with a heterogenous group of primary and secondary immune deficiency including patients with systemic lupus erythematosus, mixed connective tissue disease, vasculitis, uveitis, 22Q11 syndrome, sickle cell disease, and patients who underwent stem cell transplantation for bone marrow failure.

Findings We enrolled 69 individuals aged 14-18 years diagnosed with a primary or secondary immune deficiency in a prospective observational cohort study. All patients received a single dose of MenACWY-TT vaccine during the catch-up campaign 2018-19 because of the IMD-W outbreak in the Netherlands. Capsular polysaccharide-specific (PS) IgG concentrations against MenACWY were measured before and 3-6, 12, and 24 months after vaccination. Overall, geometric mean concentrations (GMCs) of MenACWY-PS-specific IgG were lower in patients compared to data from healthy, aged-matched controls (n=75) reaching significance at 12 months postvaccination for serogroup A and W (adjusted GMC ratios 0.26 [95% CI: 0.15 – 0.47] and 0.22 [95% CI: 0.10 – 0.49], respectively). No serious adverse events were reported by study participants.

Conclusions The MenACWY conjugate vaccine was less immunogenic in adolescent patients with primary or secondary immunodeficiency compared to healthy controls, urging the need for further surveillance of these patients and supporting considerations for booster MenACWY conjugate vaccinations in these patient groups.

INTRODUCTION

Individuals with primary or secondary immunodeficiencies are more susceptible for a severe course of infections, because of their underlying disease and the use of immunomodulating medication that compromises immune defense against infection [1]. The spectrum of primary immunodeficiencies is large and includes humoral immune deficiency (such as common variable immunodeficiencies (CVID), cellular and combined immune deficiencies (CID), complement disorders, and (functional) asplenia. Secondary immunodeficiency is often due to immunosuppressive treatment and therefore can occur in a wide spectrum of inflammatory diseases, such as juvenile idiopathic arthritis (JIA) and inflammatory bowel disease (IBD), but may also be caused by more rare diagnoses including mixed connective tissue disorder (MCTD), systemic lupus erythematosus (SLE), as well as several forms of vasculitis. Prevention of infections is crucial to reduce the number of (intensive care) admissions and mortality of invasive bacterial infections such as (vaccine-preventable) meningococcal and pneumococcal disease [2-4]. Data on immunogenicity of polysaccharide-conjugate vaccines in medical high-risk groups such as immunocompromised patients remain relatively scarce [5, 6].

In 2018, a national outbreak of serogroup W invasive meningococcal disease (IMD-W) in the Netherlands urged the implementation of a meningococcal serogroup A, C, W and Y (MenACWY) conjugate vaccination at 14 years of age, in addition to a catch-up campaign for individuals 14-18 years [7]. We conducted a prospective observational study on antibody levels before and after MenACWY vaccination in a cohort of individuals diagnosed with primary or secondary immunodeficiency aged 14-18 years, with a follow-up of two years.

METHODS

Adolescents with immune disorders from the Wilhelmina Children's Hospital of the University Medical Centre Utrecht were recruited for this prospective observational cohort study. For the current study, we enrolled patients with primary and secondary immunodeficiencies, excluding juvenile idiopathic arthritis (JIA) or inflammatory bowel disease (IBD) as these data were described separately (manuscript submitted). As part of the nationwide catch-up campaign in 2018 for individuals aged 14-18 years in the Netherlands, participants received a single dose of MenACWY-TT (Nimenrix®) from their local public health centers [7]. All patients had received primary MenC-TT

conjugate vaccine at 14 months of age according to the national immunization programme (NIP). Clinical data and blood collection were combined with routine outpatient follow-up visits. Blood samples were collected before vaccination and at 3-6 months, 12 months (+/- 3 months) and 24 months (+/- 3 months) after vaccination. We measured MenACWY polysaccharide (PS)-specific serum IgG concentrations with a fluorescent bead-based multiplex immunoassay (MIA), as previously described [8]. Data were compared with published data from healthy, aged-matched controls (HCs) who participated in a randomized controlled trial, in which antibody concentrations were determined by the same laboratory according to the same procedures [9, 10]. For safety measurements, we evaluated self-reported (serious) adverse events post-vaccination. Written informed consent was obtained from participants and caregivers (for patients <16 years).

Patient characteristics at baseline (sex, age, disease category and medication use) were presented as frequency with percentage for categorical variables and median with interquartile range (IQR) for numerical variables. For all analyses, MenACWY-PS specific IgG concentrations were log-transformed prior to analysis and presented as geometric mean concentrations (GMCs) with 95% confidence intervals (CI). In order to adjust for baseline differences, GMCs were compared between study participants and HCs at 12 months postvaccination using a multivariable linear regression analysis. The regression coefficient was exponentiated to obtain (adjusted) GMC ratios with 95% CIs for study participants versus HCs. GMCs in the total patient cohort were compared between study visits for each serogroup using pairwise t-tests with Bonferroni correction. GMCs were compared between boys and girls within the study participant cohort at all study visits using the t-test. GMCs within patients were compared between the different diseases, as well as between three large disease subgroups (primary immunodeficiency, secondary immunodeficiency, hematological condition) at all timepoints using ANOVA test. For all analyses, a p-value of <0.05 was considered statistically significant. All analyses were performed using R version 4.0.

RESULTS

We included 82 patients in the current study between October 2018 and March 2020. For 13 patients no serological data were collected and these patients were excluded from further analyses. The median age of participants was 15.3 years, and approximately half of the remaining 69 participants were female (49%) (Table 1).

Table 1. Patient characteristics at baseline

Characteristics	Total cohort (n=69)	Healthy controls (n=75)
Female, n (%)	34 (49.3%)	36 (48.0%)
Age in years, median (IQR)	15.3 (13.7 – 17.0)	15.2 (14.9 – 15.5)
Disease, n (%) N/A		
Immune deficiencies[1]	33 (47.8%)	
Autoimmune and auto-inflammatory diseases[2]	20 (29.0%)	
Uveitis	12 (17.4%)	
Sickle cell disease	4 (5.8%)	
Medication use, n (%)		
NSAIDs	5 (7.2%)	
*Immunosuppressive drugs**	23 (33.3%)	
Systemic corticosteroids	1 (1.4%)	
Synthetic DMARDs	19 (27.5%)	
Methotrexate	6 (8.7%)	
Azathioprine	1 (1.4%)	
Mycophenolate mofetil	11 (15.9%)	
Other	1 (1.4%)	
Biologic DMARDs	10 (14.5%)	
Anti-TNF	7 (10.1%)	
Anti-IL6	2 (2.9%)	
Anti-IL1	1 (1.4%)	

Abbreviations: DMARDS, disease-modifying anti-rheumatic drugs; IQR, interquartile range; N/A, not applicable. *including systemic corticosteroids, synthetic DMARDS, biologic DMARDS

[1]11 common variable immunodeficiency; 8 22Q11 syndrome; 3 chronic neutropenia; 3 specific polysaccharide antibody deficiency; 2 complement 2 deficiency; 1 bone marrow failure/allogeneic stem cell transplantation; 1 hyper IgE syndrome; 1 warts, hypogammaglobulinemia, infections and myelokathexis syndrome; 1 ataxia telangiectasia; 1 dysimmunoglobulinemia; 1 IgG subclass deficiency

[2] 6 systemic lupus erythematosus; 3 mixed connective tissue disease; 3 juvenile dermatomyositis; 2 systemic sclerosis; 1 recurrent idiopathic pericarditis; 1 chronic recurrent multifocal osteomyelitis; 1 localized scleroderma; 1 eosinophilic granulomatosis with polyangiitis; 1 alopecia areata with dysimmunoglobulinemia; 1 adenosine deaminase 2 deficiency

At baseline, GMCs of ≤0.5 µg/mL were observed for all serogroups (Table 2) with GMCs in the patients significantly lower compared with HCs for serogroup A and W, but not for serogroup C and Y (Figure 1). GMCs increased for all serogroups at three months postvaccination, the highest increase observed for serogroup C, which concerns a booster vaccination. Compared with HCs at 12 months postvaccination, GMCs were lower ($p<0.01$) for serogroup A and W (2.5 and 1.1 µg/mL versus 8.2 and 4.5 µg/mL respectively) but not significantly different for C and Y (14.6 and 2.1 µg/mL versus 16.9 and 2.5 µg/mL respectively) (Figure 1, Table 2).

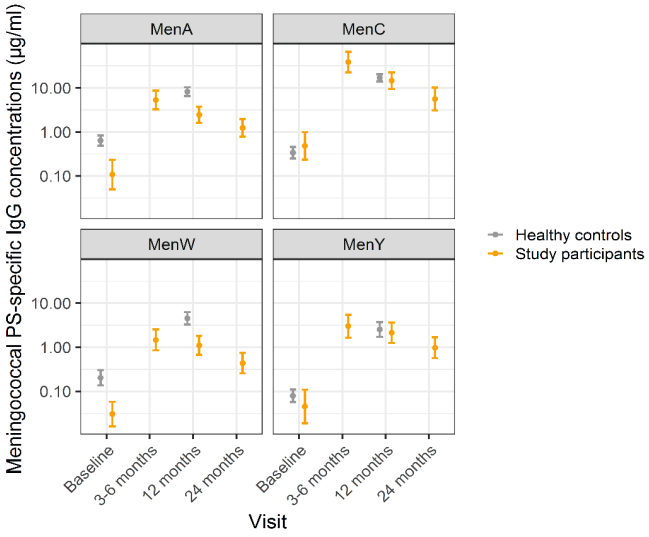

Figure 1. Geometric mean concentrations of meningococcal serogroup A, C, W and Y (MenACWY) polysaccharide (PS)-specific serum antibody concentrations of study participants and healthy controls during follow-up. Dots indicate geometric mean concentrations with 95% confidence interval.

Table 2. Geometric mean concentrations and 95% confidence intervals of MenACWY PS-specific IgG concentrations (µg/ml) during follow-up.

Months	Serogroup	Study participants	Healthy controls	*P*
0		n = 26	n = 75	
	MenA	0.1; 0.1 – 0.2[1]	0.6; 0.5 – 0.8[1]	<0.01*
	MenC	0.5; 0.2 – 1.0	0.3; 0.3 – 0.5	0.36
	MenW	0.0; 0.0 – 0.1	0.2; 0.1 – 0.3	<0.01*
	MenY	0.0; 0.0 – 0.1	0.1; 0.1 – 0.1	0.23
3-6		n = 45	n = 0	
	MenA	5.3; 3.3 – 8.7	-	-
	MenC	38.5; 22.5 – 65.8	-	-
	MenW	1.5; 0.9 – 2.5	-	-
	MenY	3.0; 1.7 – 5.5	-	-
12		n = 47	n = 75	
	MenA	2.5; 1.6 – 3.7	8.2; 6.5 – 10.4	<0.01*
	MenC	14.6; 9.4 – 22.5[1]	16.9; 14.0 – 20.4	0.53
	MenW	1.1; 0.7 – 1.8	4.5; 3.3 – 6.3	<0.01*
	MenY	2.1; 1.3 – 3.7	2.5; 1.7 – 3.8	0.60
24		n = 39	n = 0	
	MenA	1.2; 0.8 – 2.0	-	-
	MenC	5.6; 3.1 – 10.2	-	-
	MenW	0.4; 0.3 – 0.7	-	-
	MenY	1.0; 0.6 – 1.7	-	-

*$P < 0.05$

[1]one missing observation

Overall, differences between the various disease groups were limited, though patient subgroups were small (Figure 2 and Supplementary Table 4). GMCs showed a higher trend in patients with sickle cell disease compared to patients with primary or secondary immunodeficiency at 12 months postvaccination (Figure 2, $p<0.05$ for serogroup A and C, not significant for serogroup W and Y), but the sample size for each group was very small.

Two patients reported an event of special interest after vaccination: one patient reported a transient headache and another patient (who received a concomitant influenza vaccination) fainted after the vaccination with spontaneous recovery; this patient also reported a transiently enlarged lymph node. No serious adverse events were reported.

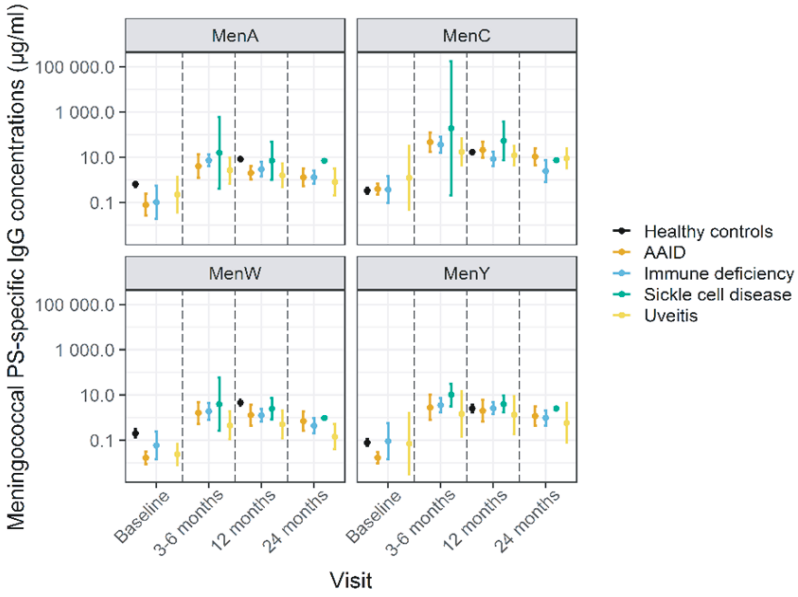

Figure 2. Meningococcal serogroup A, C, W and Y polysaccharide (PS)-specific serum IgG concentrations in patients and healthy controls during follow-up. Dots indicate geometric mean concentrations with 95% confidence intervals. Abbreviations: AAID, autoimmune and auto-inflammatory diseases

DISCUSSION

Our study found a lower IgG antibody response to a single primary MenACWY-TT conjugate vaccine in immunocompromised adolescents compared to HCs against serogroup A and W, but not for the booster vaccination to serogroup C. Serious adverse events following the MenACWY vaccination were not reported in the current study population.

To our knowledge, no studies have reported results on the meningococcal conjugate vaccine response in autoimmune inflammatory rheumatic disease (AIIRD), other than two studies on MenC conjugate vaccination in JIA patients [5, 11, 12]. Our findings are consistent with a study in JIA and IBD patients, showing a reduced functionality of antibodies and a lower proportion of protecting antibodies against serogroup W following MenACWY vaccination; differences in that study were especially pronounced in anti-TNF users (submitted). Data in literature concerning immunocompromised individuals are scarce, including groups at high risk for IMD such as individuals with complement deficiencies or use of eculizumab [13-15]. In the Netherlands, one booster is advised 3-5 years after the primary single MenACWY and MenB vaccination for asplenic individuals aged 1-24 years [16]. For complement deficiencies, repeat boosters every 5 years are advised. However, for other primary immunodeficiencies and for both pediatric and adult AIIRD patients, there are currently no recommendations on meningococcal vaccines available [17, 18]. Generally, the European Alliance of Associations for Rheumatology recommends to follow the NIP for pediatric AIIRD patients [6], but our studies suggest extra vaccination may be required for some individuals with immunodeficiency. Recommendations based on studies in the larger patient groups (JIA, IBD) are often extended to all AIIRDs because of a lack of data rather than similar immune pathology. This highlights the importance of further research into all (rare) diseases to improve protection against vaccine-preventable diseases in immunocompromised individuals and should include the immediate vaccine response as well as waning of antibodies over time as differences in kinetics have been described compared with HCs [11]. Importantly, albeit a small sample size, no severe adverse events were reported by the patients in our cohort in the first three months postvaccination. This confirms the assumption that meningococcal conjugate vaccines are safe in patients with immunodeficiencies.

Given the design of the study, we included a heterogenous patient cohort with a variety of (rare) immune disorders with low numbers for each separate disease and medication group, which hampered assessing differences between medication groups.

Because sampling was dependent on regular hospital visits, a number of serum samples were lacking at different timepoints. We only assessed serogroup-specific IgG concentrations, while addition of a functional assay [19] would have been of value because functional circulating antibodies are crucial in the prevention of IMD. A strength of the study was that we compared with data from age-matched HCs from the same laboratory and using the same procedures.

Based on our observations, it seems sensible to consider a second MenACWY-TT dose for these immunocompromised groups. This however warrants further study and follow-up of these vulnerable patient groups.

ACKNOWLEGDEMENTS

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Supplementary Table 1. Linear regression analyses for the geometric mean concentration (GMC) ratio of meningococcal serogroup ACWY polysaccharide-specific serum IgG concentrations at 12 months postvaccination for study participants versus healthy controls.

Serogroup	Analysis	GMC ratio for study participants vs. healthy controls (95% CI)
MenA	Crude	0.30 (0.19 – 0.46)*
	Adjusted[1]	0.26 (0.15 – 0.47)*
MenC	Crude	0.86 (0.57 – 1.29)
	Adjusted[1]	0.97 (0.58 – 1.61)
MenW	Crude	0.24 (0.14 – 0.42)*
	Adjusted[1]	0.22 (0.10 – 0.49)*
MenY	Crude	0.84 (0.44 – 1.59)
	Adjusted[1]	0.64 (0.23 – 1.74)

*statistically significant effect

[1]adjusted for baseline IgG concentration

Supplementary Table 2. P-values for pairwise comparisons of meningococcal serogroup ACWY polysaccharide-specific serum IgG concentrations at different study visits for all study participants.

Serogroup	Time-point	Baseline	3-6 months	12 months
MenA	3-6 months	<0.01*	-	-
	12 months	<0.01*	0.11	-
	24 months	<0.01*	<0.01*	0.28
MenC	3-6 months	<0.01*	-	-
	12 months	<0.01*	0.05*	-
	24 months	<0.01*	<0.01*	0.07
MenW	3-6 months	<0.01*	-	-
	12 months	<0.01*	1.00	-
24 months		<0.01*	<0.01*	0.08
MenY	3-6 months	<0.01*	-	-
	12 months	<0.01*	1.00	-
	24 months	<0.01*	0.05*	0.35

*$P < 0.05$

P-values were adjusted with Bonferroni correction

Supplementary Table 3. Geometric mean concentrations and 95% confidence intervals of meningococcal serogroup ACWY polysaccharide-specific serum IgG concentrations (µg/ml) for study participants during follow-up stratified by sex.

Months	Serogroup	Girls	Boys	*P*
0		n = 15	n = 11	
	MenA	0.1; 0.0 – 0.1	0.2; 0.1 – 1.1[1]	0.12
	MenC	0.3; 0.2 – 0.7	0.8; 0.2 – 3.6	0.32
	MenW	0.0; 0.0 – 0.0	0.1; 0.0 – 0.3	0.02*
	MenY	0.0; 0.0 – 0.0	0.2; 0.0 – 1.2	<0.01*
3-6		n = 25	n = 20	
	MenA	5.6; 2.8 – 11.2	5.0;2.3 – 10.7	0.82
	MenC	47.9; 20.9 – 110.1	29.3; 14.8 – 57.7	0.35
	MenW	1.6; 0.7 – 4.1	1.3; 0.7 – 2.3	0.66
	MenY	3.0; 1.2 – 7.5	3.0; 1.3 – 6.8	0.98
12		n = 24	n = 23	
	MenA	2.2; 1.2 – 4.2	2.8; 1.5 – 5.0	0.60
	MenC	19.3; 10.2 – 36.5	10.7; 5.8 – 19.9[1]	0.17
	MenW	1.2; 0.6 – 2.6	1.0; 0.5 – 2.0	0.63
	MenY	2.3; 1.1 – 4.7	2.0; 0.8 – 4.7	0.79
24		n = 18	n = 21	
	MenA	1.2; 0.5 – 2.8	1.3; 0.7 – 2.2	0.96
	MenC	7.9; 3.4 – 18.2	4.2; 1.7 – 10.2	0.29
	MenW	0.7; 0.3 – 1.7	0.3; 0.2 – 0.6	0.15
	MenY	1.2; 0.5 – 2.8	0.8; 0.4 – 1.7	0.47

*$P < 0.05$

[1]one missing observation

Supplementary Table 4. Geometric mean concentrations and 95% confidence intervals of meningococcal serogroup ACWY polysaccharide-specific serum IgG concentrations (µg/ml) in study participants reported for each disease type during follow-up.

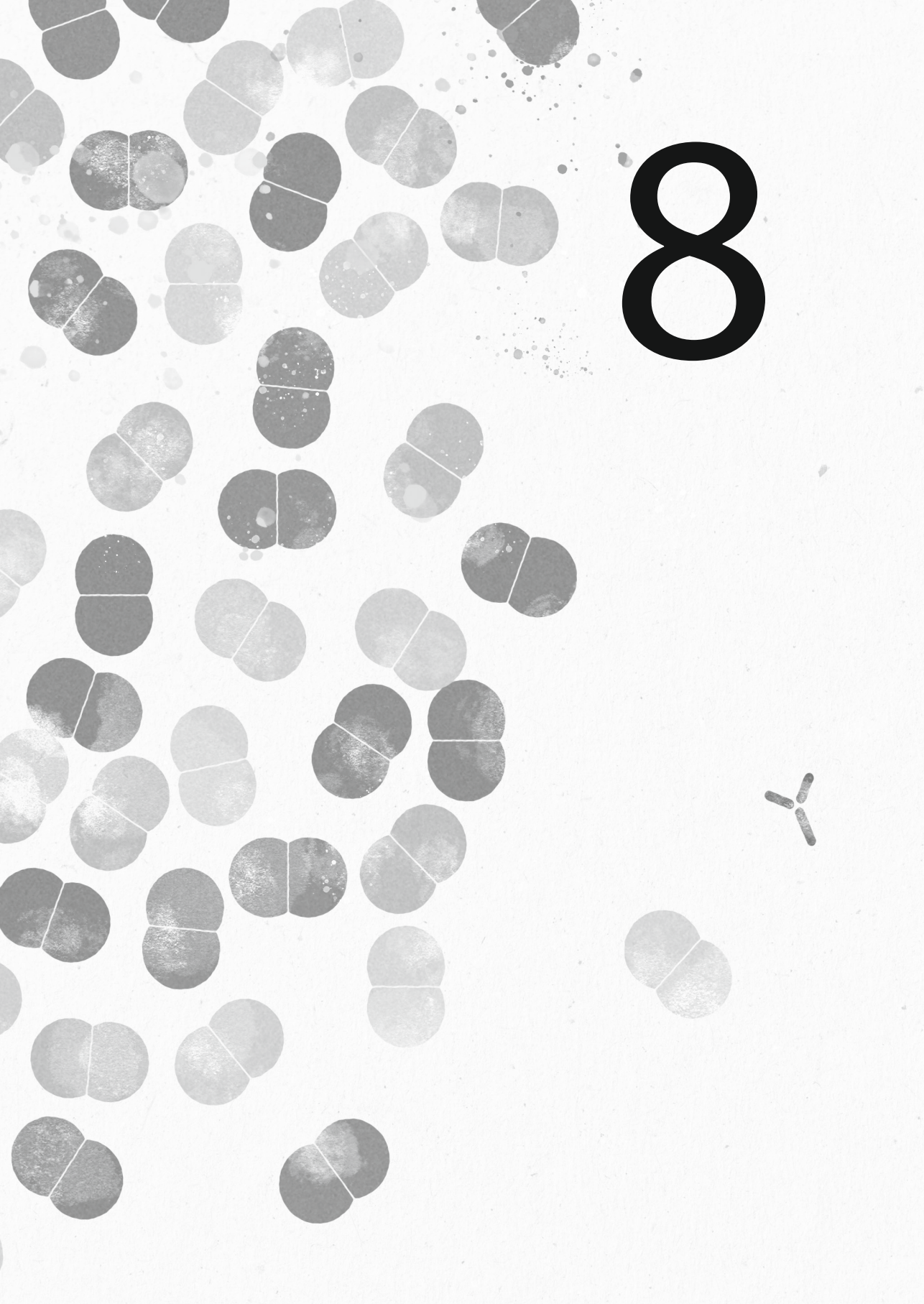
Months	Serogroup	AAID	Immune deficiency	Uveitis	Sickle cell disease	*P*
0		n = 10	n = 11	n = 5	n = 0	
	MenA	0.1; 0.0 – 0.2	0.1; 0.0 – 0.6[1]	0.2; 0.0 – 1.4	-	0.61
	MenC	0.4; 0.2 – 0.7	0.4; 0.1 – 1.5	1.2; 0.1 – 32.7	-	0.44
	MenW	0.0; 0.0 – 0.0	0.1; 0.0 – 0.2	0.0; 0.0 – 0.1	-	0.19
	MenY	0.0; 0.0 – 0.0	0.1; 0.0 – 0.6	0.1; 0.0 – 1.6	-	0.19
3-6		n = 14	n = 20	n = 8	n = 3	
	MenA	4.1; 1.2 – 13.4	7.3; 4.1 – 13.0	2.6; 0.7 – 9.9	15.8; 0.4 – 602.8	0.27
	MenC	47.2; 17.7 – 125.6	36.2; 16.2 – 80.7	17.1; 4.5 – 65.8	194.3; 0.2 – 180592.8	0.23
	MenW	1.6; 0.5 – 4.9	1.9; 0.8 – 4.4	0.5; 0.1 – 1.9	4.0; 0.3 – 59.1	0.20
	MenY	2.8; 0.8 – 10.0	3.6; 1.7 – 7.5	1.5; 0.1 – 14.6	10.1; 3.2 – 32.0	0.52
12		n = 16	n = 17	n = 10	n = 4	
	MenA	2.1; 1.0 – 4.2	3.0; 1.4 – 6.1	1.6; 0.5 – 5.2	7.1; 1.0 – 49.7	0.31
	MenC	20.9; 9.4 – 46.7	8.4; 4.1 – 17.3	12.1; 4.6 – 31.8[1]	53.3; 7.6 – 372.4	0.08
	MenW	1.3; 0.5 – 3.7	1.3; 0.7 – 2.4	0.5; 0.1 – 2.1	2.5; 0.8 – 7.3	0.35
	MenY	2.0; 0.7 – 6.0	2.6; 1.4 – 4.7	1.3; 0.2 – 9.0	4.0; 1.7 – 9.3	0.73
24		n = 14	n = 17	n = 7	n = 1	
	MenA	1.3; 0.5 – 3.2	1.3; 0.7 – 2.5	0.8; 0.2 – 3.1	7.0; 7.0 – 7.0	0.56
	MenC	10.6; 4.4 – 25.5	2.4; 0.8 – 7.5	9.1; 3.3 – 25.2	7.4; 7.4 – 7.4	0.13
	MenW	0.7; 0.3 – 1.9	0.4; 0.2 – 1.0	0.1; 0.0 – 0.5	1.0; 1.0 – 1.0	0.19
	MenY	1.2; 0.4 – 3.1	1.0; 0.4 – 2.1	0.6; 0.1 – 4.4	2.5; 2.5 – 2.5	0.78

Abbreviations: AAID = autoimmune and auto-inflammatory diseases

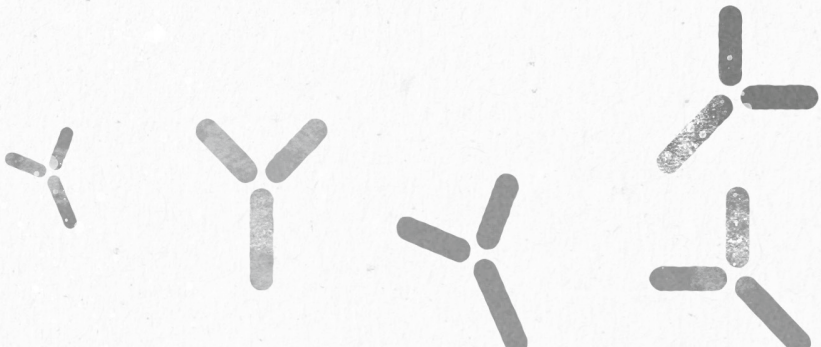
*$P < 0.05$

[1]one missing observation

8



General discussion



Invasive meningococcal disease is a much-feared, serious illness and Neisseria meningitidis *is one of the most important causes of bacterial meningitis worldwide. The case-fatality rate of IMD has been reported to be up to 25% despite appropriate medical treatment. Young children and teenagers are disproportionally affected. Meningococcal C conjugate vaccine was implemented in the national immunization programme of the Netherlands in 2002 for toddlers at the age of 14 months. The MenC conjugate vaccine was replaced by a meningococcal ACWY conjugate vaccine since 2018. Also, teenagers now receive a booster MenACWY vaccination at 14 years of age with a MenACWY conjugate vaccine. This thesis described a series of studies on vaccine-induced immunity and effect of MenACWY vaccination in several (age) groups and in the Dutch population as a whole. These studies contribute to insights for optimizing protection against meningococcal ACWY disease in the Netherlands. Here, I will summarize the findings of the research. In the general discussion I will elaborate on the most important findings of the thesis and discuss future perspectives.*

Summary of main findings

In **chapter 2**, we investigated the naturally- and vaccine-induced meningococcal seroprevalence among the population in the Netherlands, aged 0-89 years in two cross-sectional nationwide serosurveillance studies pre- and post-MenACWY conjugate vaccine implementation (2016-17 and 2020). We determined antibody levels and observed a decrease in meningococcal seroprevalence for all serogroups across most age groups nationwide in 2016-17 compared with a prior serosurveillance study in 2006-07. These results underlined the importance of the introduction of a MenACWY vaccination in the national immunization programme (NIP) upon the serogroup W outbreak in 2018 for both teenagers and toddlers.

In **chapter 3,** we examined the impact and effectiveness of the MenACWY vaccination campaign in toddlers and teenagers after the IMD-W outbreak. The vaccination campaign proved highly effective in the targeted age groups with no IMD-W cases reported in vaccine-eligible teenagers after the campaign started. Data pointed at potential indirect herd protection following the introduction, although distinguishing natural fluctuation in IMD-ACWY incidence from vaccine effect was not feasible while the coronavirus disease 19 (COVID-19) pandemic also hampered data interpretation following the campaign.

In **chapter 4**, we investigated the long-term seroprotection after a MenACWY vaccination in healthy adolescents and middle-aged adults. Antibody levels up to five years postvaccination remained high in adolescents yet had waned in part of the middle-aged adults. While adolescents - primed in early childhood with a MenC conjugate vaccine - were estimated to remain protected against serogroup C for 32 years after vaccination, the estimated duration of protection in middle-aged adults - who were not primed before - was only four years for serogroup C. The protection in adults against serogroup W and Y was estimated to last longer than for serogroup C i.e. 14 years for W and 21 years for Y, but shorter than in adolescents (up to 98 and 33 years respectively).

In **chapter 5**, potential sex-related differences to the vaccine response in healthy adolescents were investigated. A difference in geometric mean SBA titers and polysaccharide-specific IgG concentrations in favor of girls was observed. Differences were observed for 12- and 15-year-olds, but not for 10-year-olds. The percentage of adolescents that reached protective serum antibody titers was very high in both sexes and at all timepoints and proportions did not significantly differ between sexes.

In **chapter 6**, we determined SBA and polysaccharide-specific IgG vaccine responses in juvenile idiopathic arthritis (JIA) and inflammatory bowel disease (IBD) patients aged 14-18 years who received a MenACWY conjugate vaccine during the catch up campaign because of the IMD-W outbreak. We observed significantly lower antibody responses in adolescents with JIA or IBD who were treated with anti-TNF agents. Therefore it is advised to consider a second (booster) dose for those adolescent patients treated with anti-TNF to optimize protection against meningococcal ACWY disease.

In **chapter 7**, the MenACWY conjugate vaccine response was assessed in a heterogeneous group of individuals 14-18 years of age diagnosed with primary or secondary immune deficiency, who received this vaccination during the MenACWY campaign,

though excluding the most common and larger groups of JIA and IBD patients. We found lower antibody levels in patients compared to data from healthy, aged-matched controls. Again, extra doses for those patients may be considered to optimize protection against meningococcal ACWY disease.

Current situation in the Netherlands

Immunization programme and population-based seroprotection

Major steps have been made in the battle against infectious diseases, with the discovery of a smallpox vaccine in the year 1796 by Edward Jenner as the basis for vaccine development being one of the most important contributions [1]. The first meningococcal (capsular polysaccharide) vaccine was licensed in the 1970s and the later development of conjugate vaccines (capsular polysaccharide conjugated to a carrier protein) has really been a big step forward in the vaccinology field. These conjugate vaccines led to improved immune induction particularly in the very youngest as well as induced herd protection effects due to eradication of carriership. In 1999, the United Kingdom was the first country to implement a MenC conjugate vaccine in the national immunization programme (NIP) and to carry out a mass campaign during the outbreak of IMD-C. Shortly afterwards, the Netherlands followed in 2002 with a mass campaign for 1-18 year-olds and introduction of the MenC conjugate vaccine at 14 months of age in the NIP. The mass campaign that accompanied the introduction was responsible for the steep decline in IMD-C in all age groups due to eradication of MenC carriage [2]. Overall IMD incidence declined in the years thereafter [3], with a sharp vaccine-induced decline in IMD-C but also a natural decline in IMD-B with time. When the MenC vaccination was replaced by MenACWY vaccination in 2018 and a booster vaccination was implemented at 14 years of age together with a catch-up campaign for 14-18 year-olds in 2018-19, similar containment of serogroup W due to carriage eradication in teenagers was also aimed at and indeed a decline was observed in all age groups after start of the campaign (**chapter 3**). Notably, natural fluctuation could not be ruled out since the increase in incidence already had plateaued and COVID-19 measures that started in 2020 are likely to have largely contributed to the low number of IMD cases observed. During the control measures, this was not only observed for IMD but also for other invasive bacterial and viral infections [4, 5]. Close monitoring remains crucial to allow early detection of communicable disease threats after lifting of the COVID-19 restrictions. Routine childhood immunization programmes were often partly or completely postponed worldwide during the COVID-19 pandemic [6]. In the Netherlands, the consequences of the pandemic for participation in NIP vaccinations appeared to be limited and great efforts were made to vaccinate as many children as

possible [7]. In 2021, the uptake of the MenACWY vaccination in the cohort of vaccine-eligible adolescents was estimated at 85%. A comparison with previous years was not possible because this was the first year of implementation in the NIP. The coverage in toddlers was estimated at 92%, which was slightly lower (-1%) compared to the years prior to the COVID-19 pandemic. Recovery of delayed vaccinations as well as continuation of immunization in future outbreaks are of high importance to battle vaccine-preventable diseases.

While IMD-C cases have remained low since the mass campaign in 2002 –on average 6 persons suffer from IMD-C each year in the Netherlands [8]. We found that serological immunity in the whole population was low 15 years after the introduction of MenC vaccination in 2002 (**chapter 2**). In the United Kingdom, waning immunity for MenC was also observed, albeit nationwide immunity was not as low as what we observed [9]. Despite low levels of seroprotection in some age groups, no reintroduction of IMD-C was observed until 2022 in the Netherlands. This is potentially due to remaining herd protection with still low circulation of MenC in the population following the mass campaign in 2002. Yet, without an adolescent booster, the low levels of immunity in most age groups in the Netherlands might have led to a problematic increase of IMD-C in the future.

Up until recently, robust data confirming that vaccination with a MenACWY conjugate vaccine also induces herd protection was lacking. A systematic review published in 2021 assessed the effectiveness of meningococcal conjugate vaccines on meningococcal carriage and found reduced pharyngeal carriage after immunization with a MenC conjugate vaccine, but not after a MenACWY conjugate vaccine [10]. However, results from a very recent observational study from the UK indicated reduced carriage acquisition for serogroup and genogroup W and Y after the introduction of the MenACWY conjugate vaccine in the national programme which suggests that herd protection is likely [11]. Furthermore, they found ongoing low genogroup C carriage in school students 15-19 years of age. In contrast, they found no evidence that 4CMenB vaccine (Bexsero®) reduced carriage and induced herd protection. In the Netherlands, no meningococcal carriage studies were performed after introduction of the MenACWY conjugate vaccine in 2018. The most recent large carriage study that was performed among adolescents and young adults collected swabs in 2013-14 and mainly detected non-groupable meningococci, with serogroup B as most identified serogroup [12]. A carriage study - that coincided with the start of the MenACWY catch-up campaign - collected saliva and oropharyngeal swabs among 300 college students (who were not vaccine-eligible) in 2018 [13]. While incidence of IMD-W reached its peak, the

prevalence of genogroup W in carriage was still low in this specific cohort (4 out of 299 students, 1.3%). Large carriage studies with a follow-up of years are probably necessary to assess if transmission was limited through the MenACWY vaccination campaign. Disease epidemiology did show that IMD caused by serogroup A, C, W, Y cases in vaccine non-eligible groups decreased after introduction of MenACWY conjugate vaccine (**chapter 3**). Although seriously hampered by a short follow-up due to COVID-19 social distancing measures, the first observations suggested a reduction of cases in non-eligible groups. Continuous surveillance is required to see if herd protection is induced by immunization of 14-18 year-olds with a MenACWY conjugate vaccine, as occurred after the MenC conjugate vaccine in the mass campaign for all 1-18 year-olds and with a high uptake in 2002.

How herd protection via carriage eradication is induced by conjugate vaccines is still not fully unraveled, but the IgA, IgG and potentially also IgM humoral response is indispensable. With a decrease in serological and mucosal immunity, the risk of acquisition, carriage and transmission of the meningococcal bacterium increases [14]. Epidemiological patterns of meningococcal carriage and disease in the Netherlands have been highly similar to the UK over the last decades, where it was modelled that herd protection induced by the mass campaign would stabilize the incidence of IMD-C at low levels for 15 years [15]. Even though an increase in IMD-C was not observed yet in the Netherlands, we found that most age groups lacked protective IgG antibodies against serogroup C in 2016-17 (**chapter 2**), just before the introduction of the (booster) MenACWY conjugate vaccine. Furthermore, antibody protection against serogroup AWY was absent in all age groups, emphasizing the need for a (booster) MenACWY vaccination to provide individual protection and hopefully long-lasting indirect herd protection against those serogroups. Changing epidemiology influences the extent to which immunization strategies remain successful, but recent data on MenACWY herd protection encourage its use in immunization programmes to provide both direct and indirect protection for a longer time.

Factors influencing vaccine-induced antibody responses

The level of (in)direct protection in the population is influenced – through altered dynamics in transmission – by the duration of vaccine-induced protection. A longer duration of seroprotection after MenACWY vaccination was observed for MenCWY in a follow-up study in healthy adolescents (primed at young age with MenC) compared to healthy but previously MenC unvaccinated middle-aged adults (**chapter 4**). A difference in antibody responses between adolescents and middle-aged adults (50-65 years of age at time of vaccination) was already observed shortly after Men-

ACWY vaccination and was attributed to a lower IgM response in those middle-aged adults [16]. However, this finding should not deter implementation for adults if future changes in (age-specific) incidence require so. Especially when incidence rises, future (repeated) vaccinations for adults may be required to provide direct protection against IMD in adults. In the latest IMD-W outbreak, the absolute number of cases was highest in middle-aged and older adults (**chapter 3**). In addition to epidemiological surveillance on IMD cases, regular serosurveillance studies in the population are required to target groups at risk in case new outbreaks occur. A study by Borja-Tabora et al. showed that a booster dose of MenACWY-TT after the priming dose 10 years prior resulted in protective antibody levels in adults 18-55 years of age [17]. The optimal age for directly protecting older adults remains to be answered. Currently, a clinical study at the National Institute for Public Health and the Environment is investigating immunogenicity in adults aged 65-85 years of age and exploring the effect of a booster dose one year after priming. Ageing of the immune system leads to a decline in the humoral and cellular immune response, with a decreased response to (new) antigens [18]. This age-associated immune dysfunction, called immunosenescence, is associated with a reduced response to vaccination, in particular with new antigens [19]. A higher dose or regularly administering booster vaccinations is one of the approaches to improve the response to vaccination in older adults, yet booster doses might still fail to elicit sufficiently protective antibody levels at later age. Possibly, the best strategy may turn out to be the prolongation of interrupted transmission i.e. herd protection, by immunization of younger age groups (adolescents and young adults) who have the highest meningococcal carriage rates and are the main transmitters. Therefore, continuous effort to monitor, evaluate and optimize the NIP with lifelong vaccinations remains vital in particular for the most vulnerable groups like young children, elderly and immunocompromised persons.

One of the factors influencing the vaccine response that may be considered, in addition to age, is sex. Females generally have a higher antibody response while males have a higher risk of infection, although this also depends on age and hormonal status [20]. Yet, if a vaccine response is very high for both sexes, as turned out to be the case for adolescent boys and girls after a MenACWY conjugate vaccine (**chapter 5**), this does not have practical implications for an immunization programme like the NIP since nearly everyone is protected. This may become different when vaccine responses upon immunization are low, due to older age or immunosuppression. This seems to indicate that analyzing vaccine responses and reporting results not only by age but also by sex should be a standard procedure in clinical (vaccine) trials. We found a higher antibody response in girls compared to boys (aged 12-15 years) after meningococcal

vaccination. This is in line with what was previously found for influenza vaccination; the antibody response induced by a half-dose influenza vaccine in females was comparable with full-dose in males [21]. For other vaccinations, the few studies that report results according to sex found no, slight or inconsistent differences in vaccine-induced antibody levels. This stresses the need for more knowledge [22, 23]. Albeit a growing attention for sex-related differences in infectious diseases and immune responses as well as adverse events since the COVID-19 pandemic [24], it is still often ignored.

The health status may, in addition to sex and age, influence the immune response to vaccination. We investigated the antibody response after immunization with a MenACWY conjugate vaccine in a group of immunocompromised adolescent patients (14-18 years of age) diagnosed with either JIA or IBD. A lower vaccine response was observed in patients treated with anti-TNF agents and it turned out that one-fourth of the patients was not protected against serogroup W one year after a MenACWY conjugate vaccine (**chapter 6**). Therefore, we proposed that an extra booster vaccination should be seriously considered one year after the regular adolescent vaccination, to protect these patients during the period in their lives when they have an increased risk of acquisition and transmitting the meningococcal bacterium [25]. While a previous study with a MenC vaccination in JIA patients found a significant rise in MenC-specific IgG concentrations [26], they also noted lower MenC-specific IgG concentrations upon immunization with MenC conjugate vaccine in patients treated with disease-modifying antirheumatic drugs. More studies investigating the influence of anti-TNF agents on the response to a meningococcal conjugate vaccine are lacking. Results from a pneumococcal conjugate vaccine study are in conflict with what we found, and showed similar responses to vaccination in adult patients with rheumatoid arthritis treated with TNF blockers [27]. Some other studies are in line with our observation on TNF inhibitors and reduced immune responses with an important role of immunosuppressive therapy in a reduced vaccine response; a recently published prospective cohort study in adults found that biological immunomodulatory drugs impaired the response to both the 13-valent pneumococcal conjugate vaccine and the 23-valent pneumococcal polysaccharide vaccine and advised to investigate alternative strategies such as additional doses [28]. Possibly, the impaired T-helper cell response – induced by the use of immunosuppressants – requires the administration of repeated vaccine doses. In addition to drug therapy, future studies should evaluate the influence of age on the vaccine response - since this is not necessarily similar in children and adults - as well as previous exposure to vaccinations. It has been proposed that the therapeutic range of anti-TNF agents might be different in children (with a relatively higher dose necessary in children due to different pharmacokinetics of monoclonal antibodies) compared to

adults, which highlights age-related therapy differences [29, 30]. In adolescents with primary or secondary immune deficiencies, a lower antibody response to a MenACWY conjugate vaccine was also observed (**chapter 7**). This suggests that not only drug therapy, but also disease itself may influence the vaccine-induced immune response.

In summary, multiple factors such as age, sex and health status, among many others, should be taken into account when assessing vaccine responses and developing immunization strategies. The influence of these factors may differ per vaccine antigen and investigating this influence requires many studies.

Future perspectives

Vaccines

Currently, pentavalent meningococcal conjugate vaccines including serogroups ACWY combined with either serogroup X or B are developed, which fits the current epidemiology worldwide with serogroup X emerging and B of persistent concern. Localized IMD-X outbreaks were reported in several countries in Africa; a cumulative incidence of 120 cases per 100 000 was identified in one district in Burkina Faso in 2010 [31, 32]. Even though the overall burden of serogroup B is relatively low and yearly incidence rates generally do not exceed 2 per 100 000 individuals in most countries, there is substantial variation with outbreaks reported globally and a case-fatality rate between 3-10% [33]. The pentavalent NmCV-5 conjugate vaccine that targets the capsular polysaccharides of meningococcal serogroup ACWYX, was shown to elicit an immune response against the serogroup X capsule in addition to serogroups ACWY [34, 35]. As a matter of fact, a single dose of the pentavalent NmCV-5 (containing 5 µg polysaccharide of each serogroup with serogroup A and X conjugated to tetanus toxoid and serogroup C, W and Y conjugated to Cross Reactive Material [CRM]197) showed comparable or higher immune responses compared with two doses of Menactra (a MenACWY polysaccharide diphtheria toxoid conjugate vaccine containing 4 µg polysaccharide per serogroup). It did not show any safety concerns in a phase 2 trial carried out in Malian children aged 12-16 months [34]. Of note, there was no benefit of an adjuvanted vaccine formulation (with aluminum phosphate) of NmCV-5 over a nonadjuvanted formulation in these children. While several different adjuvants such as aluminum are used in vaccines, meningococcal conjugate vaccines usually do not contain an adjuvant because the conjugate vaccine already stimulates a good immune response with conjugation to the carrier protein. However, particularly for vaccines used in older adults, the addition of an adjuvant might improve efficacy of the vaccine [36]. Thus, the added value of adjuvants may very per antigen but also with age.

The pentavalent MenABCWY vaccines that are currently being developed are combined vaccines of conjugate-based and protein-based vaccines, constituted from two already licensed vaccines: for example the combination of MenACWY-TT and MenB-factor H binding protein (FHbp) developed by Pfizer [37, 38] and the combined MenACWY-CRM and 4CMenB vaccine developed by GSK [39]. First results from the Pfizer vaccine show noninferiority of the combined MenABCWY compared to MenB-FHbp alone and a comparable safety profile [38]. Although these results are promising, meningococcal protein vaccines have not been able to induce herd protection and have not been able to reach the same individual protection levels as meningococcal conjugate vaccines [40]. Since the so-called MenB vaccines target meningococcal surface structures such as outer membrane proteins rather than the serogroup-specific polysaccharide, it is sensitive for the diversity of meningococci, including changes in expression of surface proteins by circulating strains [41]. Yet, the vaccine-induced antibodies were cross-reactive against several antigen variants and antibodies that targeted different epitopes showed to have a synergistic effect on bactericidal activity [42]. The 4CMenB vaccine also provided some protection to serogroup W, although the expression of vaccine antigens on the surface influences the degree of protection that can be reached [43]. Furthermore, immunization with conjugate polysaccharide vaccines in infants and young children generally results in much higher protection levels against the targeted serogroups than meningococcal protein vaccines induce in these age groups.

To determine the optimal age for administration of a MenABCWY vaccine considering both direct and indirect protection, geographical differences and changing epidemiology should be taken into account. Serogroup B remains the major cause of IMD in Europe as well as in other continents, and serogroup W is currently the second major cause of IMD in Europe [44]. Although depending on vaccine effectiveness, implementation of the pentavalent vaccine will probably provide a broad protection against the currently most prevalent serogroups. Albeit potentially simplifying the practical aspects of vaccine schedules such as reducing the number of administrations, it must not affect the required level of vaccine-induced direct and indirect protection, which is highly dependent on the age at administration [45, 46]. The greatest burden of invasive disease in Europe in infants and young children, and highest carriage rates are observed in adolescents and young adults [25, 44]. It could be hypothesized that a MenABCWY 2+1 schedule at young age with a booster at teen age would be (most) effective to provide protection during the periods in life when an individual is most at risk for IMD. However, the current 1+1 MenACWY schedule at 14 months and 14 years of age in the Netherlands appears at the moment appears to provide protection

against those four serogroups, though we need to await the years post COVID-19 period to draw any conclusions. While introduction of the MenACWY conjugate vaccine was estimated to be cost-effective due to the high projected rise in incidence in absence of vaccine implementation during the IMD-W outbreak and the anticipated herd protection [47], it is unlikely that a MenB vaccine for infants would be as cost-effective in the Netherlands due to the currently relatively low incidence of serogroup B and the vaccine prices [48]. Furthermore, high reactogenicity of the MenB vaccine - albeit transient and of mild to moderate intensity - discouraged its use in NIPs including that of the Netherlands in the past [49, 50]. Interestingly, a study from the UK did show cost-effectiveness of MenB vaccination if spillover effects such as burden for family/caregivers and nonmedical costs were included in the analysis [51]. An altered economic evaluation of vaccines, taking into account a broader spectrum of prevention benefits, might be adopted in future cost-effectiveness methodologies [52]. Yet, whether a pentavalent MenABCWY vaccine would be cost-effective and preferable in terms of the immunization schedule and vaccine immunogenicity, as compared to separate B and ACWY schedules, is questionable.

Assays

IMD is one of the vaccine-preventable diseases for which a correlate of protection has been established: the serum bactericidal antibody (SBA) assay [53, 54]. This gold standard assay enables the assessment of vaccine-induced protection and comparison between laboratories, vaccines and immunization schedules worldwide. The SBA assay imitates the situation in case of meningococci in the blood stream, since it includes the most important components of the immune reaction (antibodies from the serum and exogenous complement) against the bacterium. This assay does not include B-cells and T cells, but while these cells are very important in the (vaccine-induced) memory response to an antigen, the fast pathogenesis requires circulating antibodies and complement that may directly act and initiate an immune response to eliminate the invading meningococci [55]. A memory response can take up to five days, while meningococcal disease is known to become quickly - within hours to a few days - fatal [56]. In meningococcal vaccine trials, there is thus a limited role of cellular assays such as the ELISpot assay that can assess (meningococcal-specific) circulating memory B-cells [57]. Yet, class-switching and generation of high-affinity antibodies follow after secondary antigen challenge [58], which emphasizes the importance of a memory B cell response *in vivo*.

An assay that could theoretically be well-correlating (or superior to) the SBA assay and used as a model to study host-pathogen interactions is the whole blood (WB) assay.

This assay uses whole blood instead of serum centrifugated from blood after collection. While the SBA assay mainly includes antibody-mediated killing and is limited to the humoral response, the WB assay also encompasses phagocytic cell-mediated killing. All the components that could possibly contribute to the immune response to invading meningococci are present in whole blood. Another advantage of the WB assay is the fact that it uses endogenous complement present in blood, therefore representing the situation in the body even more. In the evaluation of protein-based vaccines (MenB), the whole blood assay could add to determining protection levels and might even be more sensitive [59, 60]. Serogroup B might be more depending on phagocytosis than polysaccharide-specific killing due to the nature of the capsule that mimics human structure. However, the WB assay is more labor-intensive than the SBA assay because it needs to be carried out soon after collection of blood, while serum can be frozen and stored and thus also tested multiple times. While in the WB assay human complement is used, both human and baby rabbit complement can be used in the SBA assay [61]. To make the transition to animal-free science, complement from a human source is indisputably preferred over any other (animal) source of complement. However, sourcing human complement is difficult and titers established with baby rabbit complement have been proposed to correlate better with protection due to a higher sensitivity of bactericidal activity [62]. Moreover, the biological standardization that baby rabbit complement provides enables better comparison between laboratories. All in all, the SBA assay has proved to work well as gold standard and there has not been an urge to introduce a more comprehensive yet logistically more complicated assay.

A non-functional, yet less time-consuming assay that is easily used in large population studies for detecting meningococcal antibodies among others, is the multiplex immune assay (MIA) [63, 64]. This assay measures antibody concentrations by incubating antigen-specific coupled beads with serum, thereby enabling the assessment of antibody levels and distinguishing different types of antibodies such as meningococcal polysaccharide-specific IgG concentrations. Multiple antigens can be measured at once from a small serum sample (5 µl). This assay can be used for measurements in serum as well as saliva, which has previously been described to discriminate well between protected and unprotected individuals and was thus proposed as an alternative method to analyze meningococcal vaccine response [65]. However, the MIA assay is quantitative instead of qualitative (such as the SBA assay), although additional measurements of antibody avidity could add to its level of functionality. The assay is especially useful for vaccine trials, since it measures antibodies against the capsule that is targeted with vaccination, but could underestimate the protection that may

be induced by carriage (which would induce antibodies against surface proteins, that can add to bactericidal activity, in addition to the polysaccharide).

Thus, in this thesis, we combined both assays (MIA and SBA) to draw conclusions on vaccine-induced antibody responses. Even though continuous efforts to improve assays should be made, the evidence for the possible added value of the WB assay is currently not convincing enough to continue investigations into this assay further. In future vaccine trials, it would be optimal to report both SBA and MIA results. Furthermore, analyzing the impact on carriage rates could provide additional information on possible reduced colonization and transmission after immunization.

Vaccine uptake

Albeit perfectly well-working vaccines, not everyone invited to get vaccinated can seize the opportunity. During the MenACWY catch-up campaign in the Netherlands, the overall uptake was fairly high and estimated at 86% but when parents were born abroad, a lower uptake was observed [66]. For example, in adolescents with parents from Morocco the uptake was only 52% in contrast to 88% in adolescents with parents from the Netherlands. To improve protection, researchers should not only focus on the protection the vaccine induces, but also how a high and homogeneous uptake in the population may be achieved and maintained. A survey in the Netherlands showed that a strong predictor of the teenager's intention to vaccinate was the intention of the parents [67]. Communication strategies should not only target the teenager - as was widely done during the IMD-W outbreak in the Netherlands in 2016-17 via social media with the slogan "*Do not share this with your friends. Get that jab against meningococcal disease*" - but also the parents. A recently published systematic review and meta-analysis from the US found that, among others, perceiving IMD as a risk was associated with a higher meningococcal vaccine uptake [68]. This is in line with the conclusion of de Vries et al. that the focus on the severity of IMD may have played an important role in the response to the outbreak in society. An increasing positive attitude towards the MenACWY vaccination after media attention was observed, albeit it only concerned a limited discussion on safety and effectiveness of the vaccine [69]. Importantly, to increase social equity, it is essential that information is passed to all parents about this (in the Netherlands publicly funded) vaccine and the disease it prevents. Moreover, initiatives such as mobile vaccination units (buses) that go into neighborhoods with low immunization rates - to provide local residents with information on vaccination and immunize individuals without appointment - might increase the coverage, as was observed during the COVID-19 pandemic [70]. School-based interventions used for human papillomavirus (HPV)- and meningococcal campaigns were

associated with higher uptake and completion of vaccine series [71-73]. Importantly, even in high-income countries with immunization programmes that are successful, the poorest households have the highest risk of contracting the disease while also experiencing the lowest vaccination rates [74]. Developing new vaccines with high immunogenicity and long-term vaccine-induced immunity alone is thus insufficient to optimize the protection against IMD and simultaneous efforts should be made to inform and reach all individuals of the targeted groups, with easily accessible routes to get vaccinated when this is wanted.

Recommendations for risk groups

In the US, those at increased risk – including individuals with certain medical conditions such as complement deficiencies, those treated with complement inhibitors, asplenia (functional or anatomic) and HIV - for IMD are advised by the Advisory Committee on Immunization Practices (ACIP) to receive regular booster MenACWY and MenB vaccinations throughout life [45, 75]. The interval between the repeated vaccinations is different for each vaccine and also depends on the age of the patient. Furthermore, the age range for which the vaccine is licensed is different for each vaccine. For example, MenACWY-D and MenACWY-CRM are not licensed for individuals aged ≥56 years of age, and MenB vaccines only licensed for individuals 10-26 years of age. The use of these vaccines outside these age ranges is thus considered as off-label use. Several studies (mainly case reports) describe IMD in individuals receiving eculizumab despite prior immunization with a meningococcal vaccine (and sometimes also despite antibiotic prophylaxis), which highlights the difficulty of reaching protection in these patients [76-79]. However, the recommendations in the Netherlands for individuals with asplenia only advise a single MenACWY vaccination if older than 24 years of age and only 1+1 MenB vaccination when aged 16 years or older [80]. Furthermore, for many other (immune) disorders, no specific recommendations for meningococcal vaccination are formulated. For example, pediatric patients with autoimmune inflammatory rheumatic diseases were advised to follow the regular NIP based on available data [81]. Future research should investigate the effect of repeated meningococcal vaccination, as well as the timing of booster doses, among the different immunodeficiency diseases and immune modulatory medication that may be highly heterogeneous. One extra booster dose during youth might already provide long-term duration of protection throughout life for some groups, while especially those individuals who remain at high risk for IMD such as individuals with complement deficiencies would benefit from lifelong repeated immunizations. Data on IMD incidence in immunocompromised patients (including analyses on the use of immunosuppressive or immunomodulatory drugs) are often lacking, since groups are small and IMD incidence varies geographically and

with time. However, extra protection for these risk groups based on immunological data is required. Prevention by vaccination in risk groups should be investigated and continuous updates of guidelines when new data are available are necessary.

Global situation

Continuous efforts to diminish bacterial infections worldwide should be made and developed countries have an important role to play in this. Strengthening health, increasing protection against IMD and pursuing equity in the access to meningococcal vaccines should be a shared interest, even though incidence and serogroup distribution are regionally different. The World Health Organization (WHO) developed a strategy to defeat meningitis worldwide by 2030. The roadmap was established by experts to prevent meningitis through immunization, since it is still behind compared with other vaccine-preventable diseases. In the last decade, large steps have been made to counter large-scale epidemics in the meningitis belt in Africa which were an important public health threat for decades. A meningococcal serogroup A vaccine was developed through an innovative public/private collaboration by the Meningitis Vaccine Project with funding from the Gates Foundation [82]. While serogroup A accounted for the vast majority of IMD cases in the past, mass campaigns with MenAfriVac and its introduction in childhood immunization programmes led to elimination of IMD-A epidemics in the vaccination areas [83]. The advantage of the MenAfriVac vaccine was both the low cost (US $0.50, in contrast to $40-80 for comparable conjugate vaccines) and the reduced cold chain requirements [84]. A very large campaign from 2010-2016 was carried out in 26 countries in Africa with MenAfriVac and incidence of IMD-A dramatically declined afterwards. Furthermore, there were indications that indirect protection for unimmunized individuals was provided and elimination of IMD-A was almost established. However, integration of the vaccine in NIPs is indispensable for long-term persistence of these achievements [84]. At the same time in Africa, also outside the so-called meningitis belt, serogroup C and W still cause outbreaks and also outbreaks due to serogroup X have been reported [85]. A substitute for the MenA vaccine that covers these serogroups, such as the newly developed MenACWYX conjugate vaccine (with A and X conjugated to tetanus toxoid) could decrease incidence levels of IMD and, in addition, possibly increase immunity to tetanus [86].

In Europe, IMD-B is of main importance and thus a growing number of countries implemented MenB vaccination, in addition to MenC or MenACWY vaccination in infants and adolescents [87]. The United States (US) observed a large decline in IMD incidence since the recommendation of administration of a MenACWY conjugate vaccine for 11-12 year-olds in 2005 and the addition of a booster vaccination for 16 year-olds

[88]. A routine MenACWY vaccination for infants was not recommended due to the low IMD incidence in this age group. The US experienced several outbreaks of IMD on university campuses in the last decade, mainly attributed to serogroup B [89]. A MenB vaccine series was therefore recommended since 2015 for all 16-23 year-olds, rather than only college students, to control IMD-B [90]. In Australia, a programme that is generally comparable with Europa and the US is in place with MenACWY conjugate vaccine provided and MenB vaccination recommended for young children, adolescents and immunocompromised individuals [91]. In other countries of the Asia-Pacific region, a strong IMD surveillance system is lacking, which complicates monitoring of incidence. The predominant serogroups in the region are B, W and Y, and fairly high case fatality rates have been reported in some countries (up to 50% in the Philippines in infants and young children) [92]. Most countries in this region have not included meningococcal vaccinations in their immunization programmes, so there is a potential for improvements to be made in the prevention of IMD. In Latin America, serogroup B and C have been major contributors to IMD incidence, although serogroup W (especially hypervirulent strains) emerged in certain countries [93]. However, underreporting of cases remains a problem even though meningococcal disease is a mandatory notifiable disease in all Latin American countries. Limited financial resources hamper the improvement of surveillance systems that are essential to enhance meningococcal disease control [94]. Especially in countries with limited funding, it remains a challenge to defeat (meningococcal) meningitis by 2030. Ongoing efforts for a better reporting and understanding of IMD epidemiology worldwide are required to improve surveillance as well as prevention of meningococcal disease.

Conclusion

Despite the use of meningococcal vaccinations in many countries worldwide, IMD remains much-feared and can rapidly progress into a life-threatening situation despite adequate health care. The introduction of meningococcal vaccines in national immunization programmes, when implemented in addition to a mass campaign, have shown to reduce the incidence of meningococcal disease significantly for a longer period, but may require future booster vaccinations. The meningococcal conjugate vaccines provide high protection levels and with quadrivalent (and possibly in the future pentavalent vaccines) most disease-causing serogroups are targeted. Consideration of several factors such as age, sex, and health status is imperative to protect all individuals against IMD, with targeted extra immunizations for most vulnerable immunocompromised groups. Nationwide disease surveillance as well as serosurveillance are essential tools to monitor changes in epidemiology for targeted action and evolve immunization strategies during outbreaks and hyperendemic situations.

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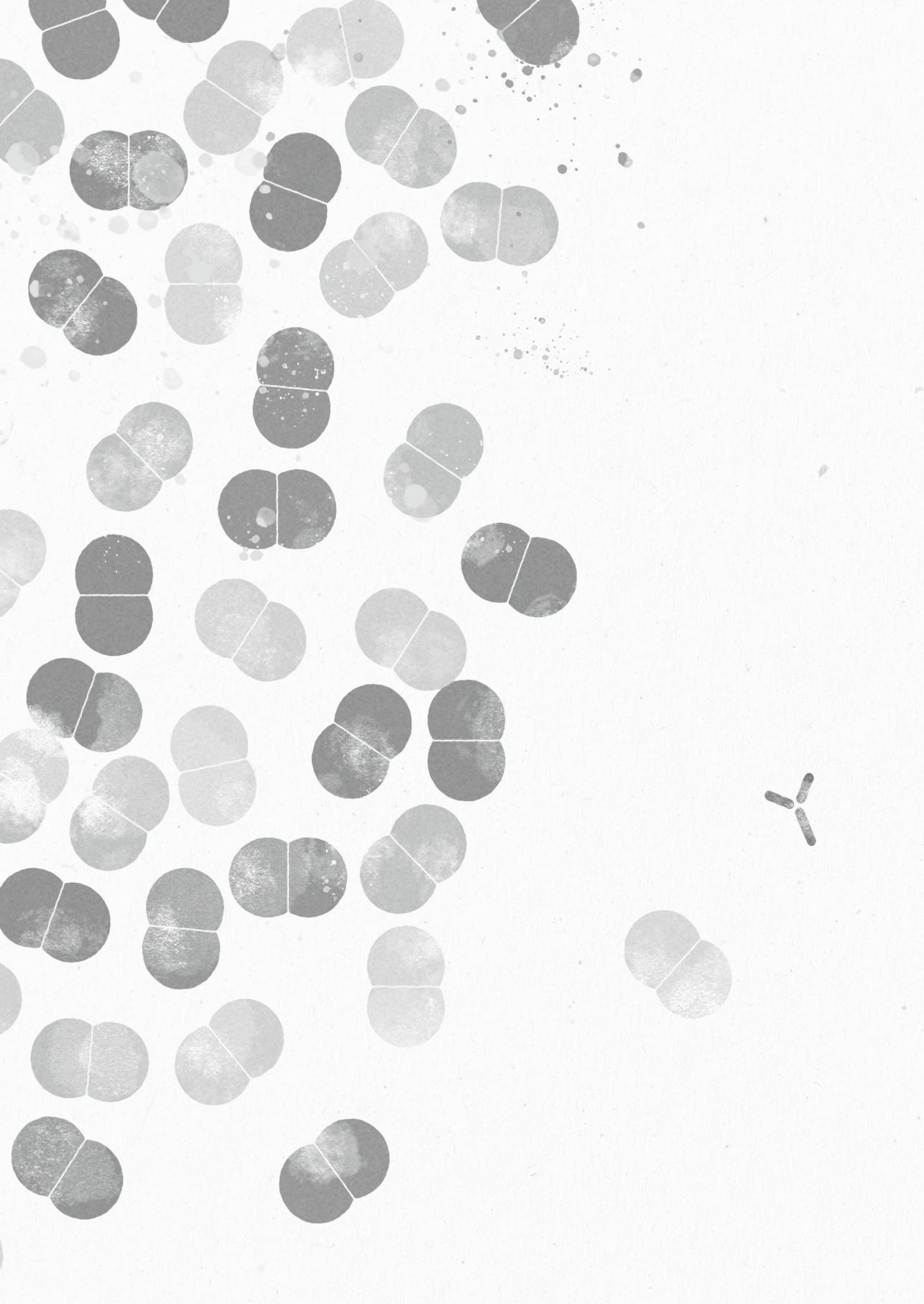


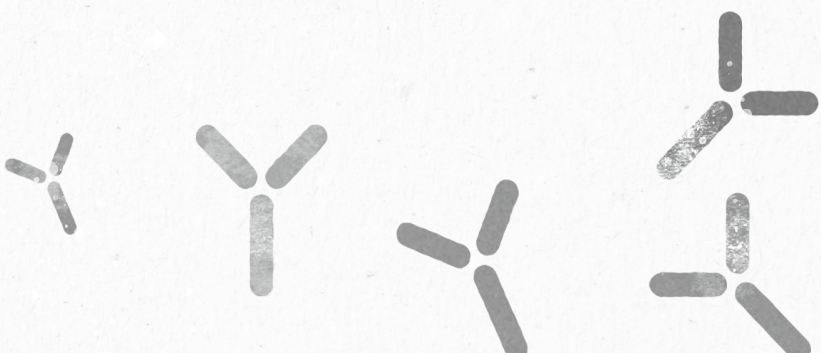
Appendix

Nederlandse samenvatting

Dankwoord

List of publications

Curriculum vitae



NEDERLANDSE SAMENVATTING

De meningokokkenbacterie zorgt wereldwijd voor ernstige, en zelfs dodelijke infecties: invasieve meningokokkenziekten. De jaarlijkse incidentie is afhankelijk van onder andere demografie, geografie en seizoen, en varieert in de tijd en per land en continent. In het verleden deden zich met name in Sub-Sahara Afrika soms tot wel 1000 ziektegevallen per 100.000 mensen voor tijdens uitbraken en ook nu nog komen daar vaak uitbraken voor. In Nederland zijn er in de afgelopen jaren meestal niet meer dan in totaal 200 gevallen van invasieve meningokokkenziekte per jaar geweest. De bacterie kan in de neuskeelholte vóórkomen bij gezonde personen zonder dat diegenen daar ziek van worden. Normaliter is dit zogenaamde asymptomatisch dragerschap van voorbijgaande aard, waarbij de kolonisatie doorgaans enkele weken tot maanden duurt. Soms dringt de bacterie echter de bloedbaan binnen en veroorzaakt ernstige ziekteverschijnselen zoals sepsis (bloedvergiftiging) en meningitis (hersenvliesontsteking) maar ook bijvoorbeeld gewrichtsontsteking (artritis), of de bacterie dringt de longen binnen en veroorzaakt een longontsteking (pneumonie). Bij aanvang van de ziekte treden vaak griepachtige verschijnselen op met koorts, maar door het snelle verloop van de ziekte kan binnen enkele uren een levensbedreigende situatie ontstaan. De mortaliteit van invasieve meningokokkenziekte ligt rond de 10% ondanks snelle en adequate antibiotica en intensive care opvang, en kan oplopen tot wel 50% bij gebrek aan medische behandeling. Bij een substantieel deel van de patiënten die de ziekte overleven, is sprake van restverschijnselen zoals slechthorend- of doofheid, epilepsie, concentratiestoornissen, of amputatie van ledematen. Gezien dit alles is preventie van meningokokkenziekte door vaccinatie cruciaal.

De meningokokkenbacterie, *Neisseria meningitidis*, is een gekapselde, gramnegatieve diplokok. Het kapsel is niet bij elke meningokokkenbacterie hetzelfde en kan uit verschillende soorten suikerketens (ook wel polysachariden genoemd) bestaan. Er zijn tot op heden 13 verschillende soorten polysacharide kapsels vastgesteld, waarbij het kapsel bepaalt welke serogroep wordt toegewezen aan de bacterie. Niet alle serogroepen zorgen voor ziekte; de (over het algemeen) meest bekende ziekmakende serogroepen zijn: A, B, C, E, W, X, Y, Z. Behalve het kapsel, dat de bacterie omringt, zijn er ook eiwitten en andere celstructuren aan het oppervlak van de bacterie die het afweersysteem van de mens kan herkennen als lichaamsvreemd, zodra de bacterie in contact komt met het afweersysteem. Als reactie hierop komen er signaalstoffen vrij en trekken witte bloedcellen naar de bacterie toe om deze in te sluiten en vervolgens af te breken; een proces dat ook wel fagocytose wordt genoemd. Dit is de eerste reactie die in gang wordt gezet door het afweersysteem en deze reactie treedt snel op, ongeacht welke

micro-organisme binnendringt. Het afweersysteem mobiliseert vervolgens B-cellen en T-cellen om een meer specifieke reactie tegen een specifieke binnendringer teweeg te brengen. De B-cellen maken met hulp van de T-cellen antistoffen aan die heel precies (een stukje van) de meningokokkenbacterie herkennen. Deze antistoffen helpen om de bacterie onschadelijk te maken. Ook zal het afweersysteem een proces in gang zetten om herkende stukjes te onthouden (geheugen B- en geheugen T-cellen), zodat er bij een nieuw contact met dezelfde bacterie snel en heel specifiek gereageerd kan worden. Op die manier heeft het afweersysteem ook een geheugenfunctie. In de bescherming tegen meningokokkenziekte kunnen antistoffen die al aanwezig zijn in de bloedbaan belangrijk zijn om ziekte te voorkomen. Zonder aanwezige antistoffen kan een meningokokkenbacterie zich snel in de bloedbaan vermenigvuldigen en daardoor ook snel voor ernstige ziekte zorgen. Aanwezige antistoffen helpen om een bacterie snel op te ruimen zodra deze het lichaam binnendringt en kunnen zo ziekteontwikkeling direct remmen. Vaccinatie zet de aanmaak van specifieke antistoffen in gang, zonder dat de bacterie aanwezig is, en is een veilige manier om bescherming te verkrijgen tegen meningokokkenziekte. Er zijn verschillende vaccins tegen meningokokken op de markt. De meeste meningokokkenvaccins bevatten de polysachariden waar het kapsel van de bacterie uit bestaat en zorgen dat er antistoffen tegen het kapsel aanwezig zijn. Als er alleen polysachariden in het vaccin zitten, worden wel antistoffen gemaakt maar geen geheugen B- en T-cellen. Door het polysacharide te koppelen aan een eiwit zoals het tetanus toxoïd eiwit, stimuleert het vaccin ook T-cellen en worden ook geheugen B- en T-cellen geïnduceerd (immunologisch geheugen). Dit soort vaccins, waarbij het antigeen wordt gekoppeld aan een dragereiwit, noemen we conjugaatvaccins. Een conjugaatvaccin gebaseerd op de kapsels van vier belangrijke typen van ziekmakende serogroepen, een meningokokken serogroepen A, C, W en Y (MenACWY) vaccin, is sinds 2018 opgenomen in het rijksvaccinatieprogramma (RVP) in Nederland, na een uitbraak in de bevolking van invasieve meningokokkenziekte veroorzaakt door serogroep W.

Voorheen was alleen een conjugaatvaccin gericht tegen de meningokokken serogroep C (MenC) in het RVP opgenomen. Dit vaccin werd in 2002 ingevoerd voor alle kinderen van 14 maanden oud vanwege een uitbraak van invasieve meningokokkenziekte veroorzaakt door serogroep C. Om de uitbraak in 2002 tegen te gaan, werden tevens alle minderjarigen van 1-18 jaar eenmalig gevaccineerd met het MenC conjugaatvaccin. Daarna was tot 2015 het aantal gevallen van invasieve meningokokkenziekte door serogroep C in Nederland (zeer) laag; er waren slechts enkele gevallen per jaar. In 2015-2016 nam het aantal invasieve meningokokken ziektegevallen opnieuw toe, dit keer veroorzaakt door een stijging in serogroep W ziektegevallen. Er werden vooral enkele

tientallen tieners ziek en ongeveer 1 op de 4 tieners met meningokokken W ziekte overleed. Om te beschermen tegen serogroep W werd de meningokokkenvaccinatie op de leeftijd van 14 maanden met het MenC conjugaatvaccin vervangen door een vaccinatie met het MenACWY conjugaatvaccin. Ook werd een herhaalprik met een MenACWY conjugaatvaccin ingevoerd voor 14-jarigen in het RVP en werden alle 14-18 jarigen (geboortecohort 2001-2005) uitgenodigd tijdens een inhaalcampagne voor een eenmalige vaccinatie met het MenACWY conjugaatvaccin.

Bescherming tegen meningokokken in de populatie

Wanneer een individu de meningokokkenbacterie bij zich draagt (kolonisatie), kan dit leiden tot de aanmaak van antistoffen tegen bijvoorbeeld het polysacharidekapsel of tegen eiwitten op de buitenkant van de bacterie. Ook na een vaccinatie worden er antistoffen tegen het polysacharidekapsel aangemaakt. Omdat deze antistoffen belangrijk zijn in de bescherming tegen meningokokkenziekte, geeft de aanwezigheid en de hoeveelheid antistoffen gericht tegen de meningokokkenbacterie in het bloed een indicatie van bescherming. De aanwezigheid en hoeveelheid antistoffen kan gemeten worden in een monster van bijvoorbeeld serum (het vloeibare deel van bloed wat overblijft nadat bloed gestold is en het stolsel afgedraaid) of speeksel. De immunogeniciteit van het meningokokken conjugaatvaccin kan worden uitgedrukt door het bepalen van de concentratie van specifieke antistoffen (meningokokken polysacharide [PS]-specifieke IgG concentratie) en de functionaliteit van alle opgewekte antistoffen in het bloed (de serum bactericide antistof [SBA] titer). Een SBA titer van 8 of hoger wordt internationaal beschouwd als de correlaat van bescherming tegen meningokokkenziekte. Deze grenswaarde is initieel vastgesteld voor de MenC bacterie, maar wordt tegenwoordig ook voor de meningokokken serogroepen A, W en Y gebruikt.
We wilden in de hele Nederlandse populatie zien of er specifieke meningokokken antistoffen tegen diverse meningokokken serogroepen aanwezig zijn en bij wie. In **hoofdstuk 2** doen we verslag van onderzoek waarbij we gebruik hebben gemaakt van drie eerder uitgevoerde, grote populatiestudies in Nederland. Bij deze zogeheten PIENTER studies is bloed afgenomen bij een dwarsdoorsnede van de Nederlandse bevolking, in de jaren 2006-2007, 2016-2017 en in 2020. In het serum is de functionaliteit van antistoffen tegen meningokokken serogroep A, C, W en Y en de concentratie van antistoffen gericht tegen deze specifieke kapsels gemeten. In 2002 ontving 94% van alle 1-18 jarigen in Nederland een MenC vaccinatie vanwege de toenmalige uitbraak door serogroep C. Na 2002 circuleert de MenC bacterie nog maar zeer weinig in Nederland en kan dus ook niet zorgen voor natuurlijke aanmaak van antistoffen. Alleen kinderen die recent gevaccineerd zijn (op de leeftijd van 14 maanden, via het RVP) en volwassenen die eerder in 2002 op de adolescentenleeftijd een MenC vaccinatie

ontvingen, bezitten nog aanwezige MenC antistoffen, zeer waarschijnlijk dankzij deze voorgaande vaccinatie met het MenC conjugaatvaccin. In vergelijking met eenzelfde soort onderzoek waarin bloed werd afgenomen in 2006-2007, zagen we in 2016-2017 een duidelijke daling in MenC antistoffen. Tegen serogroep A, W en Y werden weinig tot geen antistoffen gevonden in de hele populatie (lage seroprevalentie), wat wijst op een lage bescherming tegen deze serogroepen. De meningokokken seroprevalentie (het percentage van de mensen dat antistoffen heeft) in Nederland is dan ook in het algemeen laag.

Sinds de invoering van de MenACWY vaccinatie vanaf 2018 voor 14-18 jarigen, lijkt de gehele populatie in Nederland nu beter beschermd tegen invasieve meningokokken ACWY ziekte. Adolescenten zijn belangrijke verspreiders van meningokokkenbacterie en vaccinatie bij deze groep leidt tot minder verspreiding en daarmee bescherming van anderen in de populatie (groepsbescherming). In **hoofdstuk 3** hebben we bepaald hoe effectief de MenACWY vaccinatiecampagne in 2018-2019 voor tieners was in het verlagen van het aantal meningokokken ziektegevallen in de hele populatie. We vonden dat er een algemene daling was van 61% van meningokokken ziektegevallen door serogroep W ten opzichte van voor de campagne. In de totale groep gevaccineerde minderjarigen was de daling 82% en bij gevaccineerde tieners zelfs 100%; in die leeftijdsgroep deden zich geen serogroep W ziektegevallen meer voor na invoering van de MenACWY vaccinatie. Het is onzeker of de daling van het aantal meningokokkenziektegevallen in de hele populatie (de daling werd ook geobserveerd in ongevaccineerde groepen) alleen door invoering van de vaccinatie komt. Want ook de maatregelen tegen corona-verspreiding in 2020 hebben geleid tot daling van tal van invasieve ziekten waar we niet tegen hebben gevaccineerd. Daarnaast kunnen ook natuurlijke fluctuaties in het vóórkomen van de ziekte hebben bijgedragen aan de afname van meningokokkenziekte onder ongevaccineerden.

Afweerreactie na meningokokken ACWY vaccinatie

Al enkele jaren vóór de aanvang van de MenACWY vaccinatiecampagne in Nederland in 2018 zijn twee andere studies uitgevoerd; de zogeheten 'JIM' studie (Juveniele Immunisatie Meningokokken, onderzoek naar de optimale leeftijd voor meningokokkenvaccinatie in tieners) en de zogeheten 'StimulAge' studie (onderzoek naar het stimuleren van immuniteit op middelbare leeftijd). In deze studies werd het MenACWY conjugaatvaccin toegediend aan 10, 12, 15 jarigen (JIM-studie) en 50-65 jarigen (StimulAge-studie) en werd bloed afgenomen om de meningokokken antistoffen (PS-specifieke IgG concentraties en SBA titers) een maand en een jaar na vaccinatie te bepalen. We hebben deelnemers van beide studies vijf jaar na toediening van het vaccin

opnieuw uitgenodigd voor een bloedafname. In **hoofdstuk 4,** hebben we gekeken naar de aanwezigheid van serogroep C, W en Y antistoffen op de langere termijn, vijf jaar na het toedienen van het vaccin. We zagen dat na vijf jaar bijna alle tieners nog beschermende antistoffen (SBA titer ≥8) in het bloed hadden tegen serogroep C, W en Y. Dit was anders in de groep volwassenen (ondertussen 55-70 jaar), bij wie na vijf jaar slechts 32% nog beschermende antistoffen had tegen serogroep C, 65% tegen serogroep W en 71% tegen serogroep Y. We hebben in deze studie een wiskundig model toegepast om in te schatten hoe lang de duur van de bescherming op basis van deze data gemiddeld zou zijn. Zo konden we inschatten dat de adolescenten 32, 98, en 33 jaar beschermd zijn tegen serogroep C, W en Y na MenACWY vaccinatie (waarbij dit voor MenC een herhaalvaccinatie was, na de eerste vaccinatie op de leeftijd van 14 maanden met een MenC conjugaatvaccin volgens het RVP). In de groep volwassenen, die niet eerder waren gevaccineerd tegen meningokokken, werd dit op slechts 4, 14 en 21 jaar ingeschat voor respectievelijk serogroep C, W en Y. We denken dat zowel de vaccinatiegeschiedenis, de circulatie van de meningokokkenbacterie in de populatie, maar vooral ook de leeftijd van de deelnemers van invloed kan zijn geweest op de gevonden verschillen.

Om inzicht te verkrijgen in het effect dat het geslacht van een gevaccineerde kan hebben op de vaccinatierespons, hebben we in **hoofdstuk 5** de meningokokken PS-specifieke IgG concentraties en SBA titers na vaccinatie met MenC en MenACWY conjugaatvaccins vergeleken tussen jongens en meisjes (10-15 jaar) op verschillende tijdspunten tot drie jaar na vaccinatie. Meisjes bleken meer antistoffen te hebben dan jongens een jaar na vaccinatie, maar de verschillen waren klein. Het percentage deelnemers dat beschermd was na meningokokkenvaccinatie was voor zowel jongens als meisjes erg hoog en verschilde niet tussen de groepen. Op dit moment zouden we dan ook niet adviseren om het vaccinatieprogramma voor de meningokokkenvaccins aan te passen op basis van het geslacht. Wel is het belangrijk dat er bij toekomstig onderzoek rekening gehouden wordt met de rol die het geslacht kan spelen in de respons op vaccinatie.

Naast factoren zoals leeftijd en geslacht, kan tevens de gezondheidstoestand van een individu belangrijke invloed hebben op de vaccinatierespons. Individuen met een ziekte die gepaard gaat met een verstoord afweersysteem zijn vatbaarder voor infecties, terwijl ook de immuunreactie na vaccinatie verminderd kan zijn. Soms komt dit door de ziekte en het onderliggende verstoorde afweersysteem zelf, soms komt dit door de medicijnen die gebruikt worden bij de behandeling. Het kan per vaccin en per medicament verschillen of de vaccinatierespons al dan niet verlaagd is bij

deze patiënten ten opzichte van gezonde individuen. In **hoofdstuk 6** hebben we een studie uitgevoerd bij immuungecompromitteerde tieners met twee veelvoorkomende aandoeningen met een verstoorde afweer: JIA (juveniele idiopathische artritis) en IBD (inflammatoire darmziekte). Er deden 226 deelnemers mee, waarvan een derde leed aan IBD en twee derde de diagnose JIA had. Deze patiënten waren hiervoor onder behandeling in het Wilhelmina Kinderziekenhuis (Utrecht) en kwamen in aanmerking voor vaccinatie omdat zij tot het geboortecohort 2001-2005 behoorden tijdens de MenACWY vaccinatiecampagne. De deelnemers ontvingen de MenACWY vaccinatie bij de GGD. Het merendeel van de patiënten gebruikte medicijnen, zoals corticosteroïden (geneesmiddelen afgeleid van bijnierschorshormonen die invloed hebben op het afweersysteem) en/of biologicals (geneesmiddelen die ontstekingseiwitten van het afweersysteem beïnvloeden). In vergelijking met gezonde controles van dezelfde leeftijd (uit de eerder genoemde JIM-studie) waren de antistofconcentraties bij de patiënten lager voor serogroep A en W. Voor serogroep C en Y vonden we geen verschil, waarbij de vaccinatie tegen MenC een herhaalvaccinatie was na een eerste vaccinatie tegen MenC op de leeftijd van 14 maanden volgens het RVP. Deelnemers die vanwege hun ziekte anti-TNF medicijnen gebruikten, een bepaald type biologicals, hadden tegen alle serogroepen lagere antistofconcentraties- en titers dan patiënten die dit niet gebruikten. Ook het percentage patiënten met beschermende antistoffen (SBA titer ≥8) was lager in anti-TNF gebruikers voor serogroep W. Na een jaar had één op de vier anti-TNF gebruikers een MenW SBA titer onder de grens van bescherming (SBA titer <8) terwijl de gezonde controles allemaal nog beschermd waren na een jaar. Dit betekent dat tieners met JIA of IBD waarschijnlijk baat hebben bij een extra vaccinatie om net zo goed beschermd te zijn als gezonde leeftijdsgenoten, zeker wanneer biologicals zoals anti-TNF worden gebruikt als medicamenteuze behandeling. Er werden binnen de groep deelnemers geen ernstige ongewenste voorvallen gemeld na de vaccinatie, zoals opleving van de ziekte. In **hoofdstuk 7** hebben we een soortgelijk onderzoek gedaan in 69 tieners met andere en meer zeldzame aandoeningen (anders dan JIA of IBD) waarbij ook de afweer is verstoord, zoals systemische lupus erythematodes, sikkelcelziekte, auto-immuunziekten als oog- of vaatontstekingen en bijvoorbeeld ook patiënten die een stamceltransplantatie hadden ondergaan. Ook zij ontvingen allen de MenACWY vaccinatie tijdens de campagne in 2018-2019. Ondanks de lage aantallen patiënten viel het op dat ook hier IgG antistofconcentraties tegen serogroep A en W in patiënten lager waren dan in gezonde leeftijdsgenoten. Opnieuw zagen we geen verschil tussen patiënten en gezonde controles in de vaccinatierespons tegen serogroep C, wat ook voor deze deelnemers een herhaalvaccinatie was na een MenC vaccinatie op de kinderleeftijd. Er waren geen deelnemers die een serieus ongewenst voorval meldden na de vaccinatie.

Hoewel het huidige meningokokken vaccinatieprogramma in Nederland erg effectief lijkt, is er aandacht nodig om de vaccinatiegraad onder jongeren hoog te houden zodat de gezondheidswinst voor de gehele populatie behouden blijft. Daarnaast richt veel onderzoek richt zich momenteel op de vaccinatie tegen meningokokken serogroep B, die nu nog de meeste ziekte veroorzaakt in Europa. Het monitoren van de meningokokken epidemiologie wereldwijd is noodzakelijk om vaccinontwikkeling op tijd aan te kunnen passen aan eventuele opkomende serogroepen, zoals serogroep X die nu opkomt op het Afrikaanse continent. Ook in Nederland blijft het toezicht houden op meningokokken ziektegevallen noodzakelijk, om de effectiviteit van het huidige RVP te blijven evalueren en om vlot te kunnen handelen bij een volgende uitbraak. Serologisch onderzoek in grote populatiecohorten kan bijdragen aan goed monitoren van veranderingen in meningokokken antistofconcentraties op populatieniveau zodat bekend is wie beschermd is en wie niet.

Dit proefschrift draagt bij aan de kennis over bescherming tegen invasieve meningokokken ACWY ziekte na vaccinatie. Het bestuderen van de vaccinatierespons naar geslacht, leeftijd en gezondheidsstatus zorgt voor een beter begrip van de werking en toepassing van meningokokkenvaccins. Samenvattend heeft dit onderzoek geleid tot meer kennis van meningokokkenimmuniteit na een MenACWY vaccinatie en bescherming op populatieniveau in Nederland.

DANKWOORD

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LIST OF PUBLICATIONS

This thesis

Ohm M, Hahné SJM, van der Ende A, Sanders EAM, Berbers GAM, Ruijs WLM, van Sorge NM, de Melker HE, Knol MJ. Vaccine Impact and Effectiveness of Meningococcal Serogroup ACWY Conjugate Vaccine Implementation in the Netherlands: A Nationwide Surveillance Study. Clin Infect Dis. 2022 Jul 6;74(12):2173-2180. doi: 10.1093/cid/ciab791.

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Other publications

The National Immunisation Programme in the Netherlands : Surveillance and developments in 2020-2021: DOI 10.21945/RIVM-2021-0055

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Rivera B, Gayden T, Carrot-Zhang J, Nadaf J, Boshari T, Faury D, Zeinieh M, Blanc R, Burk DL, Fahiminiya S, Bareke E, Schüller U, Monoranu CM, Sträter R, Kerl K, Niederstadt T, Kurlemann G, Ellezam B, Michalak Z, Thom M, Lockhart PJ, Leventer RJ, Ohm M, MacGregor D, Jones D, Karamchandani J, Greenwood CM, Berghuis AM, Bens S, Siebert R, Zakrzewska M, Liberski PP, Zakrzewski K, Sisodiya SM, Paulus W, Albrecht S, Hasselblatt M, Jabado N, Foulkes WD, Majewski J. Germline and somatic FGFR1 abnormalities in dysembryoplastic neuroepithelial tumors. Acta Neuropathol. 2016 Jun;131(6):847-63. doi: 10.1007/s00401-016-1549-x.

CURRICULUM VITAE

Milou Ohm was born on 7 February 1993 in Hilversum, the Netherlands. In 2010, she finished her secondary school at the Goois Lyceum in Bussum. Subsequently, she started her bachelor studies Medicine at the Vrije Universiteit (VU) in Amsterdam. After obtaining her bachelor's degree, she took a semester off to travel through Southeast Asia. In 2014, she continued her master studies Medicine at the VU. She performed her scientific internship on 'Germline and somatic *FGFR1* abnormalities in dysembryoplastic neuroepithelial tumors' at the Royal Children's Hospital in Melbourne, Australia, under the supervision of dr. Paul Lockhart and dr. Richard Leventer. Here, she discovered her enthusiasm for laboratory work.

Her last internship took place at the pediatrics department in the Onze Lieve Vrouwe Gasthuis (OLVG West) in Amsterdam, after which she continued as a resident in pediatrics (ANIOS) there in October 2017. In December 2018, she started as a PhD candidate at the Centre for Infectious Disease Control of the National Institute for Public Health and the Environment (RIVM). Her supervisors were dr. Guy Berbers, dr. Mirjam Knol and prof. dr. Lieke Sanders. The research of her PhD focused on the vaccine-induced immunity and protection against meningococcal ACWY disease in the Netherlands. The results obtained during her PhD are described in this thesis.

In December 2022, Milou started working as a resident in medical microbiology (AIOS) at the Amsterdam University Medical Center in Amsterdam.