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Comparison of antibody response between boys and girls after infant and childhood vaccinations in the Netherlands

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ABSTRACT

Background: Infectious diseases can differ by sex in their incidence, prevalence, or severity of disease. These differences may be induced by sex-dependent immune responses and resulting protection, for example after vaccination. Therefore, this study aims to assess possible sex-differences in immunoglobulin levels (IgG) after infant and childhood vaccination.

Methods: Data from a national cross-sectional serosurvey conducted in 2006/2007 were used (Pienter 2). We compared IgG levels against measles, mumps, rubella, diphtheria, tetanus, poliomyelitis, pertussis, *Haemophilus influenzae* type b (Hib), and *Neisseria meningitidis* serogroup C (MenC) between girls and boys both short term (1 month to 1 year) and long term (1–3 year) after infant and childhood vaccinations, using linear regression analysis. Proportions of boys and girls reaching a protective IgG level were compared using Fishers exact test.

Results: Differences in IgG were found at specific time points after vaccination against measles, mumps, rubella, MenC, and polio. The geometric mean concentration or titer (GMC/T) girls:boys ratios ranged between 1.10 for polio type 1 <1 year after the first childhood booster to 1.90 for MenC <1 year after infant vaccination, indicating higher antibody levels in girls. No significant differences were found between boys and girls for diphtheria, tetanus, pertussis, and Hib at either time point. Proportions with protective levels differed only at 1–3 years after infant vaccination for mumps (82.5% boys vs. 91.9% girls, p = 0.046), and at the same time point for MenC (7.0% boys vs. 18.2% girls, p = 0.015), and polio type 1 (87.8% boys vs. 95.9% girls, p = 0.047).

Conclusion: Differences in IgG between boys and girls were generally small and not consistent, neither between pathogens nor within pathogens. If differences were observed, girls were favored over boys. On the whole, the results suggest that there are no major sex differences in protection from the studied pathogens in the Netherlands.

1. Introduction

Immune responses and protection against pathogens are known to vary between individuals depending on intrinsic and environmental factors [1]. Sex is one of the important intrinsic factors in this, as is also evidenced by differences in infectious disease incidence. For example pneumococcal disease and meningococcal disease have a higher incidence among boys, while girls suffer from pertussis more often [2,3]. Moreover, disease severity, morbidity and mortality rates can vary by sex as well; the mortality rate for measles and diphtheria are higher for females compared to males [2,4].

Possible explanations for differences in infectious diseases and immune responses between the sexes include genetic factors, environmental influences, but also sex hormones such as estradiol and testosterone, which have an enhancing or suppressive influence on the immune system, respectively [2,5]. All these factors related to sex could also induce sex-dependent responses on vaccination, as evidenced by research on early measles vaccine at 4.5 months of age, which suggested elevated immune responses in girls [6]. Similar results were found after smallpox vaccination in adults (age 18–40), with a higher antibody response in females compared to

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males [7]. Whether this also means that girls benefit from better protection than boys after infant vaccination is not yet elucidated.

Up to now, the National Immunization Program (NIP) of the Netherlands is uniform for all infants. The question arises whether we should vaccinate boys and girls differently in order to protect them equally. Therefore, this study explores whether there are differences in immunoglobulin G (IgG) levels after infant and childhood vaccinations between boys and girls, using data from a nationwide serological survey conducted in the Netherlands (Pienter 2 study). Hence, serology was used as a surrogate for protection, providing an indication for the need to intervene on the current NIP. We compared IgG levels against measles, mumps, rubella, diphtheria, tetanus, poliomyelitis, pertussis, *Haemophilus influenzae* type b (Hib), and *Neisseria meningitidis* serogroup C (MenC) between girls and boys on short term and on long term after infant and childhood vaccinations.

2. Materials and Methods

2.1. Study design

The Pienter2 study (P2) was conducted in the Netherlands between February 2006 and June 2007 as a population-wide cross-sectional serosurvey. The aim of the study was to establish a national serum bank to monitor protection and antibody levels against infectious diseases included in the NIP. A more detailed study description can be found elsewhere [8,9]. Briefly, the Netherlands were divided in five regions; eight municipalities from each region were randomly sampled to participate in the study, taking into account the number of inhabitants (40 municipalities in total). People aged 0–79 were invited to fill in a questionnaire and to donate a blood sample. In total, 19,781 people were invited, of which 6348 were willing to participate (participation rate = 32%). All participants completed a questionnaire and provided supplementary blood samples which were all tested for antibodies against different pathogens. The P2 project was approved by the medical ethics testing committee of the foundation of therapeutic evaluation of medicines (METC-STEG) in Almere (clinical trial number; ISRCTN 20164309). For children younger than 14 years of age, a parent or guardian filled in an adjusted questionnaire. Moreover, informed consent was signed by a parent or guardian prior to study participation for children younger than 11 years. Above this age, a signature of both parent or guardian and the child was required.

2.2. Study population

For our analyses only a subset of the participants of Pienter2 were included; Pienter 2 is a cross-sectional survey in which all participants were sampled at different ages and thus at different times since vaccination. Therefore, we included different cohorts of children to be able to study both IgG levels from children that were sampled between 1 month and 1 year (1m–1y) after vaccination (short term) and 1–3 years (1–3y) after vaccination (long term) based on their vaccination date. These time points were studied both after infant and childhood vaccinations (all criteria and the number of children per time point are included in Table 1). Moreover, to minimize the influence of age at vaccination on the results, for the infant vaccination we used the distribution of 'age at vaccination' from children ≤4 years of age and only included children that were in the 5–95th percentile of this distribution (i.e. a range of approximately 4–5 months). For the childhood vaccinations, we used age cutoffs that were more flexible, as there is more variation in the age at which these vaccines are administered. We included children that were vaccinated maximally one year before or after the recommended age according to the NIP. As children were included based

Table 1
Number of included children per pathogen and time point with corresponding inclusion criteria.

	Age at (last) infant vaccination according to NIP	Number of samples taken 1m–1y after infant vaccination[1]	Number of samples taken 1–3y after infant vaccination[1]	Age at first and second childhood booster according to NIP	Number of samples taken 1m–1y after first childhood booster[1]	Number of samples taken 1–3y after first childhood booster[1]	Number of samples taken 1m–1y after second childhood booster[1]	Number of samples taken 1–3y after second childhood booster[1]
Measles	14 months	84 (13–17 m)	225 (13–17 m)	9 years	86 (8–10y)	155 (8–10y)	n.a.	n.a.
Mumps	14 months	84 (13–17 m)	225 (13–17 m)	9 years	86 (8–10y)	160 (8–10y)	n.a.	n.a.
Rubella	14 months	84 (13–17 m)	225 (13–17 m)	9 years	86 (8–10y)	155 (8–10y)	n.a.	n.a.
Diphtheria (Dtx)	11 months	88 (10–14 m)	201[2] (10–14 m)	4 years/9 years	104 (3.5–5y)	161[2] (3.5–5y)	74 (8–10y)	150 (8–10y)
Tetanus (Ttx)	11 months	88 (10–14 m)	201[2] (10–14 m)	4 years/9 years	104 (3.5–5y)	161[2] (3.5–5y)	74 (8–10y)	150 (8–10y)
Polio type 1,2,3	11 months	88 (10–14 m)	201[2] (10–14 m)	4 years/9 years	104 (3.5–5y)	161[2] (3.5–5y)	74 (8–10y)	151 (8–10y)
Pertussis (Ptx, FHA, prn)[3]	11 months	66[3] (10–14 m)	190[2,3] (10–14 m)	4 years	106 (3.5–5y)	141 (3.5–5y)	n.a.	n.a.
Haemophilus influenzae type b (Hib)	11 months	86 (10–14 m)	203 (10–14 m)	n.a.	n.a.	n.a.	n.a.	n.a.
Neisseria meningitidis serogroup C (MenC)	14 months	89 (14–17 m)	224 (14–17 m)	n.a.	n.a.	n.a.	n.a.	n.a.

n.a. = not applicable.
[1] In brackets are the ages in months/years between which the child should have received the vaccine in order to be included.
[2] Children were excluded if they had already received the next booster dose.
[3] From 2005 onwards, the pertussis component in the DTP-IPV combination was changed from whole cell to acellular. Therefore, in the selection of 1m–1y after infant vaccination only children who received acellular vaccine were included, and in the 1–3y selection only whole cell vaccinees were included.

on their age and date of vaccination, they were automatically included in the right analysis (short or long term) and therefore compared in their respective birth and vaccination cohorts.

2.3. Laboratory methods

Serology samples of P2 were tested for antibodies against a broad range of pathogens; as our analyses were limited to the vaccine preventable diseases (VPD) against which a vaccine was in the NIP at the time of study conduct, we only describe the corresponding assays below. To determine IgG levels, serological testing for antibodies against the different pathogens was performed with a fluorescent bead-based multiplex immunoassay (MMRV-MIA) using Luminex technology for simultaneous detection of measles, mumps, and rubella antibodies [10]. For MenC and Hib, antibody concentrations were determined likewise in a combined assay [11]. Antibodies against pertussis, diphtheria, and tetanus were determined simultaneously using a pentaplex MIA [12]. Finally, polio IgG total antibodies were measured in a standard neutralization test (NT) [13]. IgG concentrations were determined and calibrated to internationally generally accepted standards, such as the cut-off criteria of the World Health Organization (WHO).

2.4. Vaccination data

Antibodies that were examined in this study are all directed against vaccine preventable diseases (VPD) targeted by the NIP of the Netherlands at time of data collection. As the NIP has changed over time, children that participated in P2 and that were included in these analyses, were eligible for vaccination according to the following schedules: For children from birth cohort 1993–1998, the DTP-IPV vaccination was administered at 3, 4, 5, and 11 months together with the vaccine against Hib. Childhood boosters were provided at 4 and 9 years of age for DT-IPV. From birth cohort 1999 onwards, the infant DTP-IPV and Hib vaccinations were administered at 2, 3, 4, and 11 months. From birth cohort 2005 onwards, the pertussis component in the DPT-IPV infant combination vaccine was changed from whole cell to acellular. For all included birth cohorts, at age 14 months, children received the MMR vaccine. A childhood booster against MMR was provided at 9 years of age. For birth cohorts from 1998 and later, a booster dose using acellular pertussis vaccine was added to the program at 4 years of age. For children born on or after June 1, 2001, a MenC vaccination at 14 months of age was added [14,15].

During data collection participants were asked to bring their vaccination-registration booklet, which is used to register the date and type of received vaccination. These data were checked and if necessary completed using the electronic national immunization register 'Praeventis' [16]. The 15th of the month was used as the date (day). In case of a missing month, December 15 was used as the date.

2.5. Data analyses

Geometric mean IgG concentrations or titers (GMC/Ts) with 95% confidence intervals (CI) were calculated per pathogen for boys and girls separately. In all analyses, the survey design with five strata (regions) and 40 clusters (municipalities) was taken into account, to adjust the standard error of the estimates for the sampling design.

Linear regression analyses were performed to study the association between sex and the logarithmically transformed antibody levels against the different pathogens for the different time points. Characteristics, in which significant differences between boys and girls were found, were considered for inclusion in the model to examine whether these affected the antibody levels (Table 2 and 3). We presented GMC/T ratios with 95% confidence intervals comparing girls to boys. In addition, the proportion of protected boys and girls at the different time points were compared by a Fischer's exact test using published correlate of protection cutoffs [17–23]. P-values < 0.05 were considered significant. All analyses were conducted in SAS 9.4.

Table 2
Characteristics of vaccinated children aged approximately 0–4 years (all children included at time points 1m–1y and 1y–3y after infant vaccination).

	Boys (n = 186)	Girls (n = 183)	p-value
Ethnicity, *n (%)*			0.26
Dutch	123 (66)	131 (72)	
Other	63 (34)	39 (28)	
Urbanization, *n (%)*			0.59
Very high	44 (24)	37 (26)	
High	83 (44)	51 (37)	
Moderately high	22 (12)	21 (12)	
Low	15 (8)	19 (11)	
Very low	22 (12)	21 (14)	
Parental education, *n (%)*			0.51
High	87 (47)	79 (43)	
Intermediate	79 (42)	89 (49)	
Low	15 (8)	13 (7)	
Missing	5 (3)	2 (1)	
Household income, *n (%)*			0.90
High	47 (25)	41 (22)	
Intermediate	82 (44)	87 (48)	
Low	21 (11)	20 (11)	
Missing	36 (20)	35 (19)	
Persons in household, *n (%)*			0.78
1–2	8 (4)	6 (3)	
3	36 (19)	42 (23)	
4	98 (53)	88 (48)	
≥5	42 (23)	46 (25)	
Missing	2 (1)	1 (1)	
Attending daycare, *n (%)*			0.02
Yes	131 (70)	110 (60)	
No	55 (30)	70 (38)	
Missing	0 (0)	3 (2)	
Median age in months at infant vaccination for children ≤4 years (5th–95th percentile)			
DTP-IPV	12 (10–13)	12 (10–14)	0.50
MMR	15 (14–16)	15 (13–17)	0.35
Hib	12 (10–13)	12 (10–14)	0.87
MenC	15 (14–17)	15 (14–17)	0.53

3. Results

Of 19,781 people invited for the study, 6348 were willing to participate. In the current analyses 435 boys and 450 girls were included, with the exact number of children varying per pathogen and per time point (Table 1). General characteristics of vaccinated children are listed in Tables 2 and 3, which do not show major differences between boys and girls except for daycare attendance (children < 4 years, p-value 0.02) and parental educational level (children 4–12 years, p-value 0.02). These tables also include information about the distribution of 'age at vaccination'. GMC/Ts for boys and girls at the different time points after vaccination are displayed in Supplementary Fig. 1.

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.vaccine.2019.06.055.

In the GMC ratio plots, girls were compared to boys (ratio > 1 indicates a higher level in girls). For MMR, GMCs were generally higher in girls than in boys with GMC ratios ranging from 1.00 for to 1.67 (Fig. 1). Significant differences were found for measles 1–3y after childhood booster (GMC ratio: 1.36, 95% CI 1.06–1.73),

Table 3
Characteristics of vaccinated children aged approximately 4–12 years (all children included at time points 1m–1y and 1y–3y after childhood vaccination).

	Boys (n = 249)	Girls (n = 267)	p-value
Ethnicity, *n (%)*			0.11
Dutch	176 (71)	171 (64)	
Other	73 (29)	96 (36)	
Urbanization, *n (%)*			0.42
Very high	49 (20)	66 (25)	
High	122 (49)	122 (46)	
Moderately high	27 (11)	31 (11)	
Low	31 (12)	23 (9)	
Very low	20 (8)	25 (9)	
Parental education, *n (%)*			0.02
High	97 (39)	103 (38)	
Intermediate	137 (55)	130 (49)	
Low	13 (5)	33 (12)	
Missing	2 (1)	1 (1)	
Household income, *n (%)*			0.77
High	52 (21)	52 (20)	
Intermediate	113 (45)	132 (49)	
Low	34 (14)	37 (14)	
Missing	50 (20)	46 (17)	
Persons in household, *n (%)*			0.48
1–2	7 (3)	10 (4)	
3	28 (11)	36 (13)	
4	108 (43)	119 (45)	
≥5	104 (42)	102 (38)	
Missing	2 (1)	0 (0)	
Median age in months at booster for children 4–12 years (5th–95th percentile)			
DT-IPV1	48 (43–79)	48 (42–70)	0.61
Pertussis	47 (44–60)	47 (43.5–53)	0.25
MMR	106 (15–117)	107 (18–117)	0.82
DT-IPV2	107 (56–116)	107 (61–118)	0.89

but not 1m–1y after booster or at any time point after infant vaccination. For mumps a difference was seen at 1–3y after infant vaccination (GMC ratio: 1.67, 95% CI 1.28–2.18), but not shortly after vaccination or after booster. Also for rubella, a difference was seen 1–3y after infant vaccination (GMC ratio: 1.34, 95% CI 1.08–1.66). For MenC, GMCs were significantly higher in girls than in boys 1m–1y after vaccination (GMC ratio: 1.90, 95% CI 1.09–3.34), but not 1–3y after vaccination. Adjusting for daycare attendance and parental educational level did not substantially change the results and we therefore present crude GMC ratios only.

There were no significant differences between boys and girls found for diphtheria, tetanus pertussis, and Hib. GMC ratios ranged from 0.75 (1–3y after Hib vaccination) to 1.58 (1m–1y after Hib vaccination) (Fig. 2). For polio, GMTs were generally higher in girls than in boys with significant differences at some time points, although the differences were small (GMT ratios ranging from 1.01 for polio type 3 (1m–1y after second childhood booster) to 1.21 for polio type 3 (1–3y after first childhood booster)) and not consistent across time points.

Even though we found some differences in IgG levels, the proportion reaching a protective IgG level was generally high in both boys and girls (Supplementary Fig. 2). We found three instances where boys and girls differed in the percentage having a concentration associated with protection; 82.5% of the boys had protective IgG levels against mumps 1–3y after infant vaccination, compared to 91.9% of the girls (p-value: 0.046). This coincides with the time points where a significant difference in IgG concentration against mumps was observed. In addition, we found 7.0% of the boys to reach a protective IgG level against MenC 1–3y after infant vaccination, compared to 18.2% of the girls (p-value: 0.015). Also for polio type 1, a difference was observed (girls 95.9% protected versus boys 87.8% protected, p-value 0.047). However, IgG levels against both MenC and polio type 1 did not differ significantly at that time point, which could be explained by more variation in IgG level among girls compared to boys.

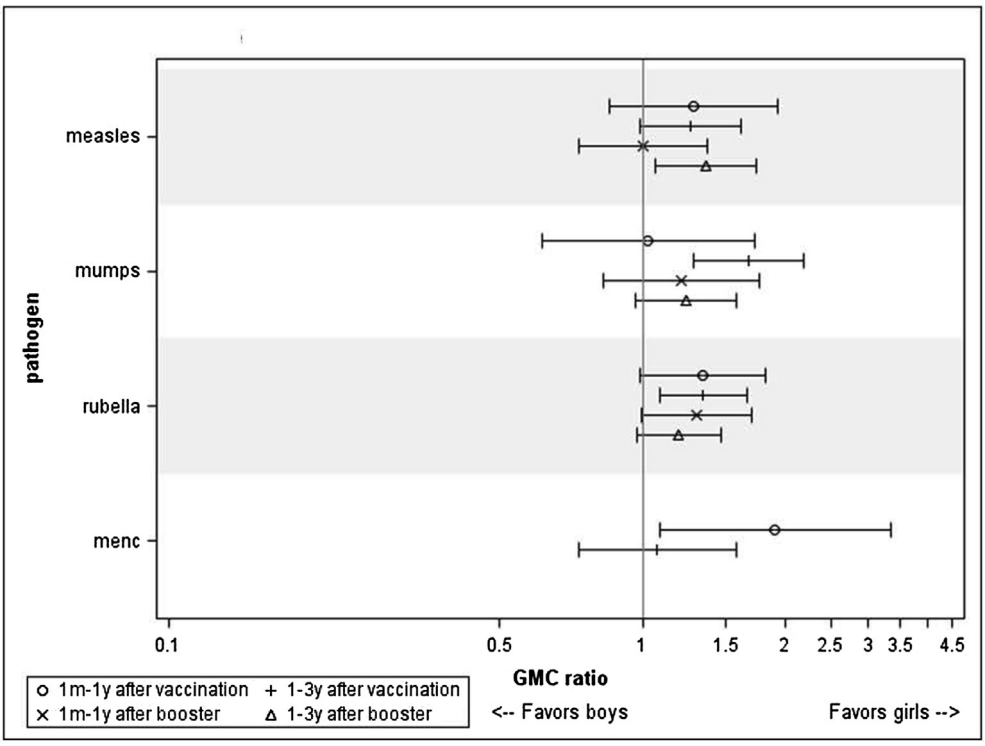

Figure 1. GMC ratios (girls versus boys) with 95% CI for measles, mumps, rubella, and MenC at different time points.

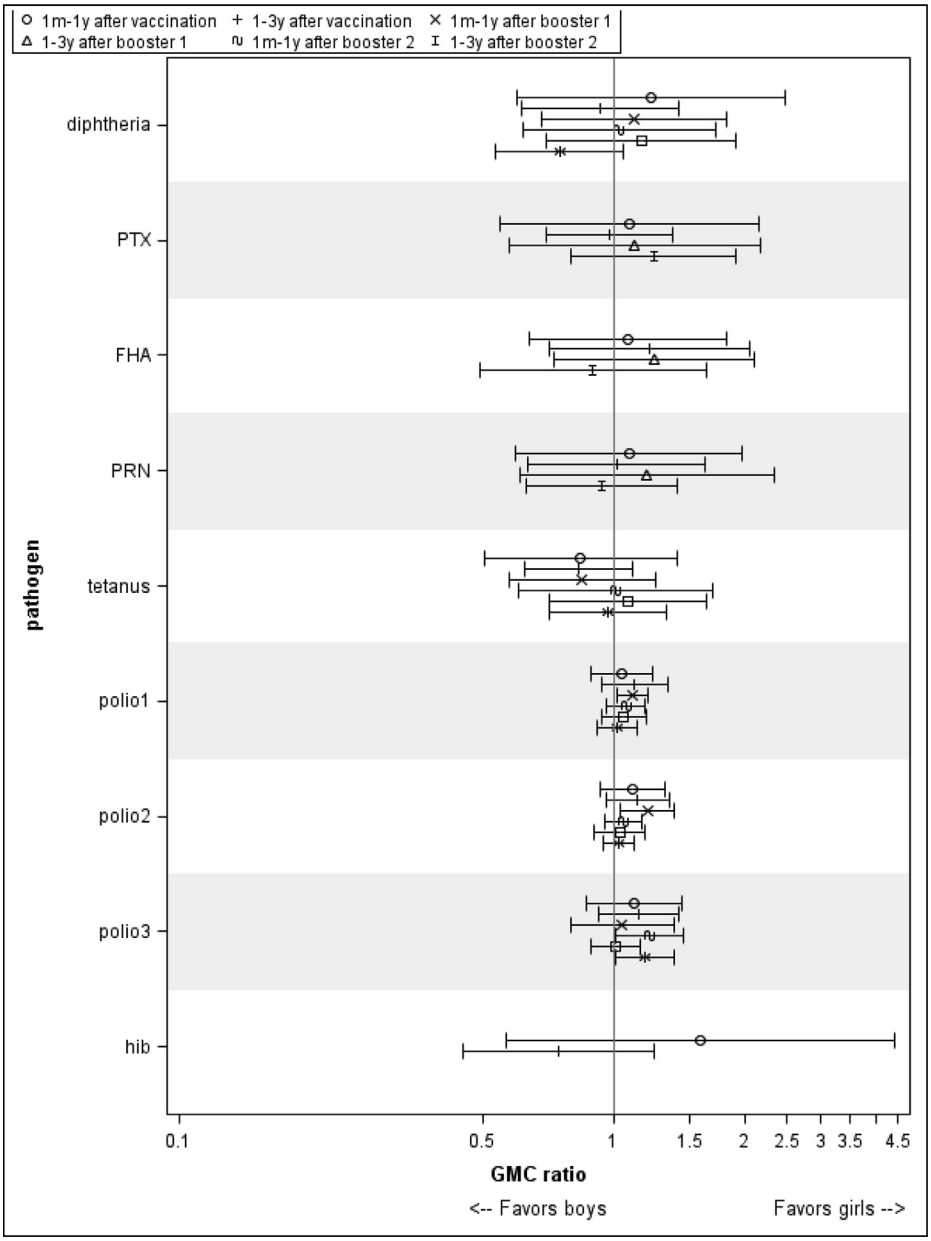

Figure 2. GMC/T ratios (girls versus boys) with 95% CI for diphtheria, pertussis, tetanus, polio, and HiB for the different time points.

4. Discussion

In this study, the association between sex and IgG levels against different pathogens after infant and childhood vaccination was assessed. For measles, mumps, rubella, MenC, and polio we found that girls had significantly higher GMC/Ts at specific time points after vaccination, with GMC/T ratios ranging from 1.10 to 1.90. We did not find such differences for diphtheria, tetanus, pertussis, and Hib. Only for mumps, MenC, and polio type 1 we found a lower proportion of boys reaching protective IgG levels. Although some differences were found, these were not consistent across pathogens and within pathogens over time points. If differences were observed, girls were favored over boys.

Evaluating immune responses after vaccination for boys and girls separately is not considered common practice, although it could be very useful. There are some studies addressing sex differences when studying vaccine effectiveness in RCTs, nonspecific effects, or immune responses [24,25]. Most of these previous studies support our results in the sense that girls are often found to have a stronger immune response than boys after vaccination, both humoral and cellular [6,7,26]. Also in a recent meta-analyses on infant vaccination the positive trend towards girls was seen; Voysey et al. indicated higher immune responses in girls than in boys after diphtheria, meningococcal A, W, and Y, and pneumococcal vaccinations [27]. These findings are in line with our results; we observed higher GMCs for girls, more specifically 1–3y after the MMR infant vaccination, 1m–1y after the MenC infant vaccination, and higher GMTs for girls at multiple time points after polio vaccination. In addition, Voysey et al. did not find an association for Hib and tetanus, which is in accordance with our results.

Both in our and previous studies, girls seem to have higher IgG levels but not consistently across all pathogens. This indicates a biological phenomenon; possible underlying mechanisms include differences between boys and girls in the sex-hormone milieu [28], or differences in immune genes located on the X-chromosome [27]. Furthermore, we hypothesize that the observed

differences might be related to vaccine type; especially the live attenuated MMR vaccine seems to show differences, as well as the conjugated polysaccharide MenC vaccine and the inactivated polio vaccine [29]. The differences in IgG levels could suggest that the aforementioned vaccines induce sex-differential effects, while vaccines containing antigen proteins do not (i.e. diphtheria, tetanus, and pertussis) [29]. However, if an explanation is to be searched in the nature of the vaccine, differences between boys and girls might be expected for Hib (conjugated polysaccharide) as well, but we did not find an association there.

The pattern reflecting sex-differential IgG levels is not consistent across time points within pathogens, indicating that a deviation at one time point does not necessarily mean that this deviation is always present. This was also seen before in a rubella re-immunization study, where adolescent boys showed higher IgG levels 2 weeks after vaccination compared to girls, but not after 10 weeks [30]. Perhaps some of our findings are reflecting natural fluctuation, or differences between memory responses are involved [26]. Therefore, it is important to keep in mind that the time points reflect different processes (i.e. after infant vaccination or after booster, short term or long term) and that different children are compared over time (no longitudinal data).

Although there are no reasons to suspect large deviations in seroprevalence in the Netherlands [14], we did examine the proportion of children reaching an IgG level that is considered protective, to see whether this would indicate sex differences. As we only found three instances where girls showed higher protection levels (mumps, MenC, and polio type 1, all 1–3y after infant vaccination), we assume that, even when GMC/T ratios show (small) differences, antibody levels are still high enough to ensure proper protection. In the three instances where we found indications for sex differences in protected proportion, it is likely that the vast majority of the infants are still benefiting from herd immunity [27]. However, in the light of current decreasing vaccination coverage in many industrialized countries, this remains an important topic for surveillance. Moreover, for MenC the SBA titer is considered more informative in terms of protection compared to IgG levels, but data on SBA titers were only available for a small sample of participants (36–40%) in our study.

Strengths of the current study include the use of a national sample from which IgG levels against multiple pathogens could be studied simultaneously. In addition, the registration of vaccination date enabled accurate selection of children who received the vaccine within the period we wanted to study. Limitations that should be addressed include the fact that multiple measurements of one individual were included in the analyses, which might be correlated, i.e. a child with a high response against measles will possibly also have a high response against rubella. This increases the change of false-positive findings. Hence, we were careful in drawing conclusions from the results and mainly looked at patterns in the data. Furthermore, it could have been interesting to evaluate clinical data on measles, mumps, and rubella disease to see whether these show differences between boys and girls, as differences in antibody levels for these pathogens were among our most consistent findings. However, due to the limited number of cases this would be difficult to do. Also from literature, limited evidence is available regarding differences in clinical disease between girls and boys or men and women. Finally, we are assuming that the immune response we are measuring is merely vaccine derived, while children could also have naturally acquired antibodies due to exposure to the pathogen. However, it seems unlikely that this would happen at a different rate for boys and girls.

In conclusion, we found some instances where IgG levels after infant and childhood vaccination differed with higher GMC/Ts among girls. However, differences were generally small and inconsistent both between and within pathogens, as all GMC/T ratios ranged from 0.75 to 1.90 and as they were hardly reflected in the protected proportion. Therefore, we have no indications to assume that there are substantial differences between boys and girls in IgG levels after vaccination that would cause unequal protection. This is a reassuring message, as currently all children in the Netherlands are receiving the same vaccinations according to the NIP. In order to continue proper monitoring of sex dependent vaccine responses, future studies on vaccine effectiveness or NIP evaluations could consider stratified analyses by sex.

Declaration of Competing Interest

The authors declare that they have no competing interest.

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Contributors

JH and MK analyzed the data, MK and FvdK designed the study, LM was involved in data collection. All authors interpreted the data and critically revised the manuscript. All authors read and approved the final manuscript.

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