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The immunogenicity and safety of a tetravalent measles-mumps-rubella-varicella vaccine when co-administered with conjugated meningococcal C vaccine to healthy children: A phase IIIb, randomized, multi-center study in Italy[☆]



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ABSTRACT

Introduction: Multiple vaccination visits and administrations can be stressful for infants, parents and healthcare providers. Multivalent combination vaccines can deliver the required number of antigens in fewer injections and clinic visits, while vaccine co-administration can also reduce the number of visits. This non-inferiority study was undertaken to evaluate the feasibility of co-administering a combined measles-mumps-rubella-varicella (MMRV) vaccine with conjugated meningococcal C (MenC) vaccine in a large cohort of healthy Italian toddlers.

Methods: Healthy subjects aged 13–15 months were randomized (2:1:1) to receive single doses of either: co-administered MMRV + MenC at the same visit (MMRV + MenC group); or MMRV followed 42 days later by MenC (MMRV group); or MenC followed 42 days later by MMRV (MenC group). Blood samples were collected before and 43 days after vaccination. Antibody titers against MMRV were measured using ELISA. Functional-anti-meningococcal-serogroup activity (rSBAMenC) was assessed using a serum bactericidal test. Solicited local and general reactions were recorded for up to 4 and 42 days post-vaccination, respectively. Non-inferiority of MMRV + MenC to MMRV (post-dose-1 seroconversion rates) and MMRV + MenC to MenC (post-dose-1 seroprotection rates) was achieved if the lower limit (LL) of the 95% confidence interval (CI) for the group difference was $\geq -10\%$ for each antigen.

Results: 716 subjects were enrolled in the study. At 42 days post-vaccination, the MMRV seroconversion rates were 99.3% (measles), 94.5% (mumps), 100% (rubella) and 99.7% (varicella) in the MMRV + MenC group, and 99.4%, 93.2%, 100% and 100%, respectively, in the MMRV group. The seroprotection rates against rSBA-MenC were 98.3% in the MMRV + MenC group and 99.3% in the MenC group. Non-inferiority was reached for all the vaccine antigens. The safety profiles were as expected for these vaccines.

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Conclusion: The immune responses elicited by co-administered MMRV + MenC were non-inferior to those elicited by MMRV or MenC alone and support vaccination of children with both vaccines at a single visit.

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1. Introduction

In Italy, from 2011 to 2015, the annual number of reported measles cases fell from 5181 (8.5/100,000 population) [1] to 247 (0.4/100,000) [2], but notification rates are still higher than in many other European countries [2]. Data from 2010 to 2011 showed that, in Italy, the vast majority (96%) of measles cases were in people who were either unvaccinated or incompletely vaccinated; and that adolescents and young adults were most affected (i.e. those that did not receive childhood measles-mumps-rubella (MMR) vaccination as part of the national immunization program, as it was only included in 1999) [3]. The uptake for MMR is particularly concerning, as $\geq 95\%$ uptake is necessary to interrupt endemic transmission of measles [2]. However, Italian data from 2014 show MMR vaccine uptake levels of approximately 87% (first dose) [4] and 83% (second dose) [5].

A two-dose varicella (V) universal vaccination program has been implemented in selected Italian regions. First-dose vaccination uptake is around 51–84% by age 2 years [4]. However, uptake was lower in regions where MMR + V was used rather than measles-mumps-rubella-varicella (MMRV) [6]. Conjugated meningitis C (MenC) vaccine uptake in the second year of life is also suboptimal in Italy, at approximately 75% [4].

As a maximum of two vaccine injections per visit is considered ideal in Italy [7], a potential tool to increase vaccine uptake is to use combination vaccines along with co-administration. The current Italian vaccination recommendations for children aged 13 months include: (1) MMRV or MMR + V; (2) MenC or conjugated meningococcal ACWY (MenACWY) vaccine; and (3) meningococcal B (MenB) vaccine [8]. Thus, co-administration of MMRV plus a meningococcal conjugate vaccine at one visit might be beneficial. However, information on the co-administration of MMRV and conjugate meningococcal vaccines is sparse. To date, only two studies have examined the co-administration of MMRV with vaccines against MenC: either meningococcal ACWY-tetanus toxoid conjugate vaccine (MenACWY-TT) [9] or MenACWY-CRM [10]. Both studies found that the vaccines could be co-administered without affecting the immunogenicity or safety profiles of either vaccine [9,10].

The aim of the study reported here was to provide data on the co-administration of MMRV and MenC in Italy. This non-inferiority study was therefore undertaken to compare the immunogenicity and safety of co- and separate administration of MMRV and MenC vaccines in healthy Italian toddlers.

2. Materials and methods

2.1. Study design and subjects

Open phase IIIb, randomized, study conducted at 13 centers in Italy between February 2012 and March 2014. Healthy male or female subjects, aged 13–15 months, were randomized (2:1:1) to receive either single doses of MMRV and MenC vaccines administered concomitantly as separate injections on the same day (MMRV + MenC Group); a single dose of MMRV at one visit followed 42 days later by a single dose of MenC vaccine (MMRV Group); or a single dose of MenC at one visit followed 42 days later by a single dose of MMRV vaccine (MenC Group).

Subjects were excluded if they had: received any investigational drug/vaccine 30 days before the study vaccine or immunosuppressive medication/immunoglobulins/blood products for ≥ 14 days within 6 months; major congenital defects; chronic illness or a family history of immunodeficiency; human immunodeficiency virus; symptoms of acute illness or fever; previous vaccination/history/known exposure to MMRV and/or *Neisseria meningitidis* serogroup C diseases.

The study adhered to Good Clinical Practice, the Declaration of Helsinki, and local Italian rules and regulations. Independent ethics committees and/or institutional review boards at each participating center reviewed and approved all study-related documents. Parents/guardians of subjects provided written informed consent before any study-related procedures were performed.

2.2. Study objectives

The co-primary objectives of the study were to show the non-inferiority of: co-administering MMRV vaccine with MenC conjugate vaccine compared to MMRV vaccine alone; and co-administering MenC vaccine with MMRV compared to MenC conjugate vaccine alone. Secondary objectives included the 42-day post-vaccination immunogenicity and tolerability of each study vaccine.

2.3. Study vaccines

A single 0.5 mL dose of MMRV vaccine (*Priorix-Tetra*TM, GSK, Belgium; batch numbers: AD01B698A, AD01C132D, AD01B538B, A71CA677A, AMRVA138A, AMRVA200B, and AMRVA162B; comprising a lyophilized cake which was reconstituted with the diluent from a pre-filled syringe before administration) was administered subcutaneously in the deltoid region of the upper arm. A single 0.5 mL dose of MenC CRM-197 conjugated vaccine (*Meningitec*, Nuron Biotech; batch numbers: F32046, F15126 and G26516) was administered intramuscularly into the upper thigh. The needle sets provided for MMRV and MenC vaccines were 8 mm 25G5 and 1.25 mm 25G, respectively.

2.4. Immunogenicity assessment

Blood samples were collected before and 43 days after vaccination. Antibody titers against MMRV were measured using commercial enzyme-linked immunosorbent assays (ELISA) – (*Enzygnos* Dade Behring, Marburg, Germany) with cut-off values of 150 mIU/mL (measles), 231 U/mL (mumps), 4 IU/mL (rubella) and 25 mIU/mL (varicella). Anti-meningococcal serogroup activity (rSBA-MenC) was measured using an in-house serum bactericidal test (cut-off: $\geq 1:8$). For measles, mumps, rubella and varicella, subjects who were seropositive for each assay prior to vaccination did not contribute to the seroconversion rate or geometric mean concentration (GMC) calculations.

2.5. Safety/reactogenicity assessment

Parents/guardians used diary cards to record solicited injection site symptoms for 4 days after each vaccine and solicited general

symptoms for 43 days post-MMRV vaccination (fever, rash/exanthema, parotid/salivary gland swelling, signs of meningism, febrile convulsions) and for 15 days post-MenC vaccination (drowsiness, irritability/fussiness, loss of appetite).

Unsolicited symptoms were recorded for 43 days after each dose and serious adverse events (SAEs) were recorded throughout the study.

The intensity of all symptoms was graded on a scale of 0–3; where “Grade 0” was none/as usual, “Grade 1” was mild, “Grade 2” was moderate and “Grade 3” was severe. Grade 3 solicited symptoms were defined as: pain: cried when limb was moved or a spontaneously painful limb; redness and swelling: injection site surface diameter >20 mm; fever: rectal temperature >39.5 °C; rash: >150 lesions; irritability/fussiness: crying that cannot be comforted/prevented normal activity; drowsiness: which prevents normal activity; loss of appetite: not eating at all. Unsolicited symptoms (including SAEs) were defined as grade 3 if they prevented normal daily activity.

2.6. Statistical analysis

All statistical analyses were performed using Statistical Analysis Software (SAS) version 9.2.

A sample size of 720 subjects was planned (MMRV + MenC: 360; MMRV: 180; MenC: 180), considering an approximately 10% non-inferiority margin for all antigens. The 2:1:1 blocking randomization scheme ensured that the balance between treatments was maintained.

The non-inferiority of MMRV + MenC versus MMRV vaccine alone was met if the lower limit (LL) of the standardized asymptotic 95% confidence intervals (CIs) for the post-vaccination difference in percentage of seroconverted subjects between the two groups (MMRV + MenC minus MMRV) was $\geq -10\%$ for each vaccine antigen. Similarly, for MMRV + MenC versus MenC vaccine, the post-vaccination difference in the percentages of seroprotected subjects between the two groups (MMRV + MenC minus MenC) was $\geq -10\%$ for rSBA-MenC antibodies.

The immunogenicity analysis was performed on the according-to-protocol (ATP) cohort, which included all subjects with available pre- and post-vaccination serology results, who were seronegative for at least one vaccine antigen and complied with study procedures. Seroconversion rates (antibody concentration/titer \geq cut-off value in initially seronegative subjects), seroprotection rates (rSBA-MenC antibody titer $\geq 1:8$), GMCs and geometric mean titers (GMTs) against each vaccine antigen were calculated with 95% CIs (cut-offs given above).

The safety analysis was performed on the total vaccinated cohort (TVC) which included all vaccinated subjects.

3. Results

3.1. Demographics

Of 716 enrolled subjects (MMRV + MenC: 351; MMRV: 183; MenC: 182), 626 were included in the ATP cohort for immunogenicity (MMRV + MenC: 315; MMRV: 168; MenC: 143; Fig. 1).

The baseline demography did not differ by study group. The overall mean age was 13.4 ± 0.6 months and 53.7% were male. The study population was predominantly Caucasian (81.3%).

3.2. Immunogenicity

At 42 days post-vaccination, the seroconversion rates for the MMRV antigens in the MMRV + MenC group were 99.3%, 94.5%, 100% and 99.7%, respectively. In the group which received MMRV

alone, the MMRV seroconversion rates were 99.4%, 93.2%, 100% and 100%, respectively (Table 1). As the LL of the 95% CI for the MMRV + MenC minus MMRV group difference was above -10% ; non-inferiority was demonstrated (Table 1).

At 42 days post-vaccination, the seroprotection rate for rSBA-MenC in the MMRV + MenC group was 98.3% compared with 99.3% in the subjects who received MenC alone (Table 1). As the LL of the 95% CI for the MMRV + MenC minus MenC group difference was above -10% ; non-inferiority was demonstrated (Table 1).

GMCs and GMT in the MMRV + MenC group were generally similar to those in the MMRV group (Table 1). However, it should be noted that no statistical analysis of these comparisons was planned in the study protocol, and no predefined non-inferiority criteria were set, so eventual comparison of these groups should be interpreted with caution.

3.3. Reactogenicity and safety

Within 4 days of vaccination, injection site redness was the most commonly reported solicited local symptom, with 29.8% subjects (MenC site) and 27.3% subjects (MMRV site) in the MMRV + MenC group, and with 23.2% subjects for the MMRV group (Fig. 2).

Irritability/fussiness was the most frequently reported solicited general symptom during the 15-day post-vaccination period and was experienced by 55.7%, 58.8% and 49.1% of subjects in the MMRV + MenC, MMRV and MenC groups, respectively (Fig. 3). Grade 3 irritability/fussiness (crying that could not be comforted/prevented) was recorded in 8.9%, 7.3% and 4.1% of subjects, respectively.

MMRV-specific solicited general symptoms reported during the 43-day post-vaccination period included parotid gland swelling in 0.9% (MMRV + MenC group), 2.3% (MMRV group); and meningism/febrile convulsion in 0.3% (MMRV + MenC group) and 0.6% (MenC group) of subjects. The percentages of subjects who developed fever during the 43-day post-vaccination period were: 64.9% (MMRV + MenC), 64.4% (MMRV) and 37.4% (MenC). The characteristic peak in fever at 5–12 days post MMRV vaccination was observed in the MMRV + MenC and MMRV groups (Fig. 4). Very few subjects received prophylactic antipyretic medication: MMRV + MenC: 0.6%; MMRV: 0.5%; none for MenC.

Only two subjects in the MMRV + MenC group reported febrile convulsions. The first occurred 8 days after vaccination, was considered related to vaccination, but was of mild intensity. The second occurred 28 days after vaccination, was a grade 3 SAE, but was considered not related to vaccination. Rather, the investigator thought that it was caused by a viral infection, as blood analyses showed leukocytosis.

Rash was observed in 27.0% of MMRV + MenC subjects, 23.2% in the MMRV group and 8.8% in the MenC group. Varicella-like rash was observed in 1.8%, 0.6% and 0.6% subjects, respectively and measles/rubella-like rash was seen in 11.9%, 9.0% and 1.2%, respectively. Grade 3 rash (>150 lesions) was reported in $\leq 0.6\%$ subjects.

At least one unsolicited symptom was reported for 27.6% (MMRV + MenC), 33.3% (MMRV) and 22.5% (MenC) of subjects. The most common symptoms were: pharyngitis in the MMRV + MenC and MenC subjects ($n = 16$ [4.6%] and $n = 9$ [4.9%], respectively) and cough, pharyngitis and diarrhea ($n = 10$; 5.5% for each symptom) in the MMRV group.

SAEs were reported in 12 subjects (MMRV + MenC: 6; MMRV: 4; MenC: 2). None were causally related to vaccination and no subject withdrew due to SAE. There were no deaths during the study.

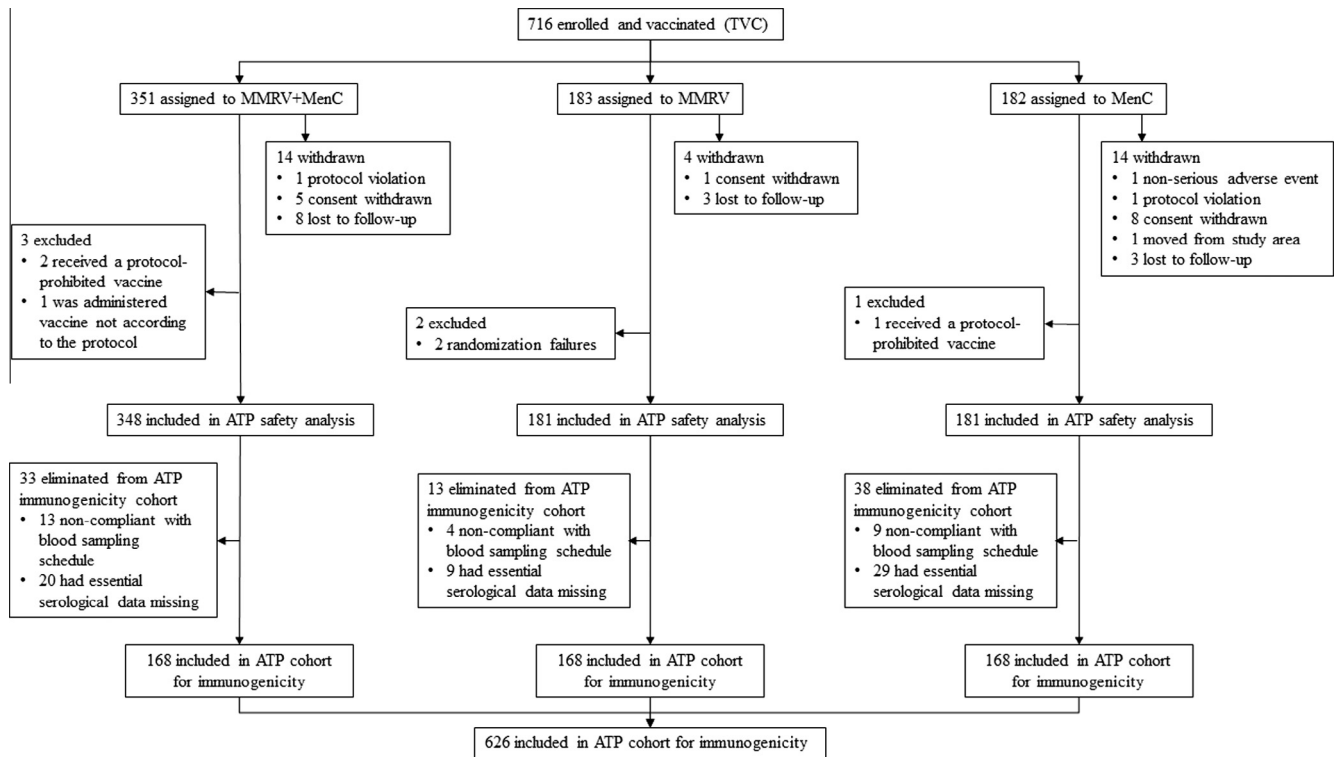


Fig. 1. Subject disposition. ATP, according to protocol; MenC, conjugated meningitis C vaccine; MMRV, measles, mumps, rubella, and varicella vaccine; TVC, total vaccinated cohort.

4. Discussion

Several trials have already shown the feasibility of co-administering MMRV with other vaccines: MenACWY-TT [9], MenACWY-CRM [10], reduced-antigen content diphtheria-tetanus-acellular pertussis-inactivated poliovirus vaccine (dTpa-IPV) or full-strength DTPa-IPV [11], 10-valent pneumococcal nontypeable *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV) [12], 4CMenB [13], or *H. influenzae* type b conjugate-hepatitis B vaccine (Hib/HepB) and diphtheria-tetanus-acellular pertussis vaccines (DTaP) [14]. However, this is the first study comparing the immunogenicity and safety of co-administered MMRV + MenC vaccines in healthy Italian children. The results show that the immune responses to MMRV and rSBA antibodies were non-

inferior after the combined administration compared with those elicited by either MMRV or MenC vaccines alone.

The immune responses to all vaccine antigens were high in all groups and were comparable to those observed by Vesikari et al. when combined MMRV + MenACWY was studied [9]. Similar results were seen in a study conducted in healthy children in the US where the MMRV vaccine was administered at the same time as other vaccines [15].

The study vaccines were generally well tolerated and clinically acceptable safety profiles were observed. The expected peak in fever within 5–12 days after MMRV dosing in our study is comparable with previous reports [16,17]. The pattern of fever was comparable between the MMRV + MenC and MMRV groups and higher than the MenC group. Similar observations were reported by Vesikari et al. [9].

Table 1
Immunological responses after MMRV + Men C, MMRV or MenC, at 42 days post-vaccination (ATP cohort for immunogenicity).

Antibody	Group	N	SC (%) (95% CI)	GMC (95% CI)	Difference in SC rate (95% CI) (MMRV + MenC minus MMRV)
Measles (≥ 150 mIU/mL)	MMRV + MenC	307	99.3 (99.7; 99.9)	2943.6 (2691.5; 3219.2)	−0.04 (−1.82; 2.78)
	MMRV	163	99.4 (96.6; 100)	3158.5 (2749.7; 3628.0)	
Mumps (≥ 231 U/mL)	MMRV + MenC	309	94.5 (91.3; 96.8)	1530.7 (1368.4; 1712.1)	1.29 (−3.04; 6.67)
	MMRV	162	93.2 (88.2; 96.6)	1591.3 (1346.2; 1881.0)	
Rubella (≥ 4 IU/mL)	MMRV + MenC	309	100 (98.8; 100)	40.2 (37.3; 43.3)	0.00 (−1.23; 2.29)
	MMRV	164	100 (97.8; 100)	44.9 (40.6; 49.6)	
Varicella (≥ 25 mIU/mL)	MMRV + MenC	300	99.7 (98.2; 100)	156.3 (143.9; 169.8)	−0.33 (−1.87; 2.03)
	MMRV	159	100 (97.7; 100)	145.2 (129.5; 162.8)	
Antibody	Group	N	SP (%) (95% CI)	GMT (95% CI)	Difference in SP rate (95% CI) (MMRV + MenC minus MenC)
rSBA-MenC (≥ 8 1/DIL)	MMRV + MenC	291	98.3 (96.0; 99.4)	491.7 (416.5; 580.4)	−1.02 (−3.39; 2.24)
	MenC	143	99.3 (96.2; 100)	586.4 (479.3; 717.6)	

N: number of subjects in each group.
 SC (%): seroconversion (percentage of subjects with titer ≥ specified value).
 SP (%): seroprotection (percentage of subjects with titer ≥ specified value).
 95% CI: 95% confidence interval.
 GMC: geometric mean antibody concentration.
 GMT: geometric mean antibody titer.

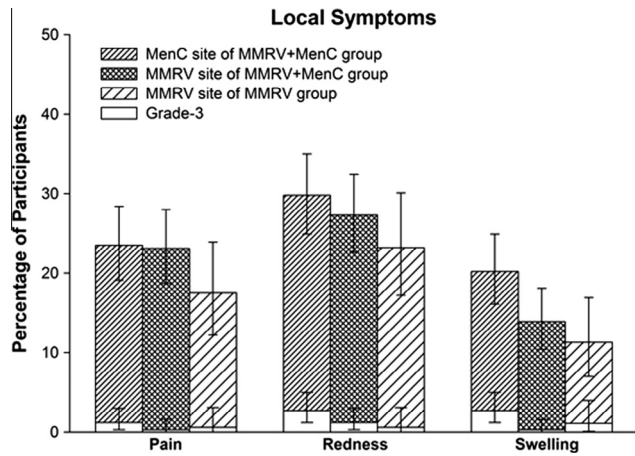


Fig. 2. Incidence of solicited local symptoms during four days after vaccination with MMRV + MenC, MMRV or MenC (total vaccinated cohort).

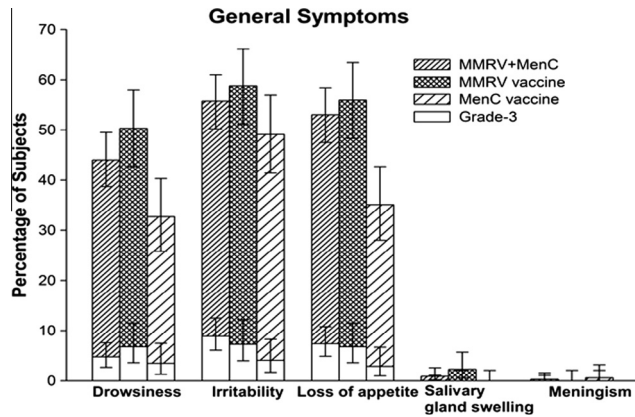


Fig. 3. Incidence of solicited general symptoms reported during the 43-day post-vaccination follow-up period (total vaccinated cohort).

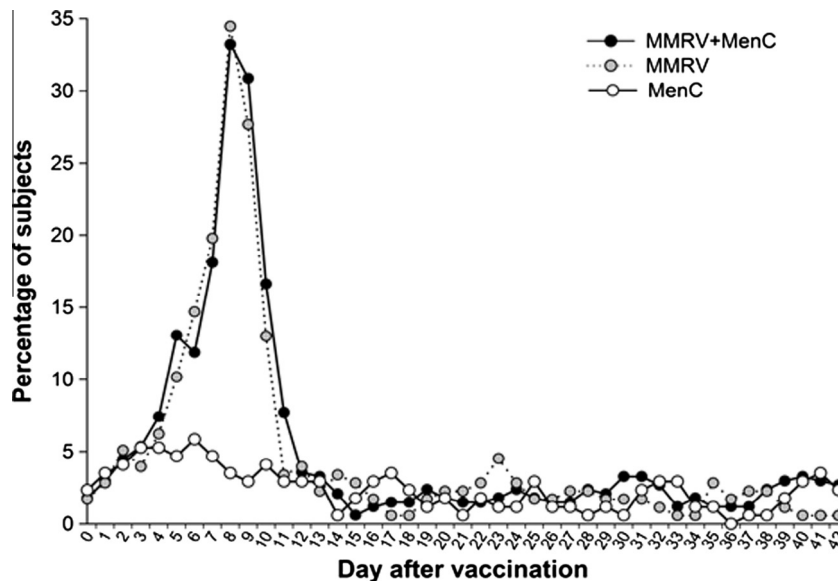


Fig. 4. Prevalence of any fever by day (days 0–42) (total vaccinated cohort).

kari et al. when ACWY-TT + MMRV and MMRV alone vaccines were given to children aged 12–23 months [9]. It is worth noting that the MMRV fever pattern was not modified by co-administration of mono- or tetravalent-conjugated meningococcal vaccines.

Although Schink et al. [18] showed a relationship between MMRV and febrile convulsion, and other studies have shown an increased risk of febrile convulsions with combined MMRV vaccine [19,20], two subjects in our MMRV + MenC group experienced a febrile convulsion. One occurred on day 8, was mild in intensity, and was considered related to vaccination; the other occurred on day 28, was a grade 3 SAE, but was not considered to be related to the vaccine. The rate of febrile convulsions observed in Germany for *Priorix-Tetra* (2.18/10,000 vaccinees) [18] is consistent with the range (0.87/10,000–3.37/10,000) recorded by the Veneto region vaccine surveillance system (Canale Verde) from 2008 to 2014 [21]. Further, a recent observational study carried out in the Veneto Region, in which over 10,000 forms were completed by mothers, showed that the overall rate of SAEs, including febrile convulsions, was no worse for MMRV as compared to MMR + V [22]. Although not statistically powered, the rate of febrile convulsion was comparable for both strategies [risk ratio (95% CI) 0.80 (0.30–2.15)]. The Veneto study also showed that mothers of toddlers who received MMR + V rather than MMRV had higher levels of stress and risk perception [22].

Measles seroconversion was high in both MMRV and MMRV + MenC groups and GMCs were also unaffected. It has been reported that GMC values for measles are higher following MMRV vaccine administration as compared to MMR + V [23].

Rash is a common adverse event following the administration of varicella vaccine, affecting between 3% and 17% of subjects [24–26]. Unsurprisingly, rash (any) was higher in the subjects who received MMRV vaccine as compared to MenC vaccine in our study. Most other adverse events were generally mild-to-moderate; and no vaccine-related SAEs were reported.

A potential limitation of this study is that it was conducted in a single country; further studies might be needed to extrapolate these results to other populations.

In conclusion, the co-administration of the MMRV vaccine with MenC was immunogenic and well tolerated in Italian toddlers. Such co-administration in routine clinical practice would reduce

the number of required clinic visits and has the potential to improve vaccine acceptance, compliance and uptake.

Trademark statement

Priorix-Tetra[™] is a registered trademark of the GSK group of companies.

Meningitec[™] is a trademark of Nuron Biotech BV, US

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Authors' contributions

All named authors have contributed in the design/acquisition of data or analysis and data interpretation. All provided substantial intellectual and scientific input during manuscript development, critically reviewing the content, revising the manuscript and giving final approval before submission. The work described was carried out in accordance with the ICMJE recommendations for conducting, reporting, editing and publishing scholarly work in medical journals.

Conflict of interest

OH, MP and FM are employees of the GSK group of companies. OH reports ownership of stock options from the GSK group of companies. PD received payments from the GSK group of companies, Crucell Berna, Sanofi Pasteur MSD, Pfizer and Novartis. MC reports personal fees and non-financial support from GSK group of companies during the conduct of the study. GG received payment from the GSK group of companies through his previous institution for conducting this clinical trial. GG acted as investigator in clinical trials for the GSK group of companies, Sanofi Pasteur MSD and Novartis, has received fees from the GSK group of companies, Sanofi Pasteur MSD and Sequirus for board membership, consulting fees from the GSK group of companies as well as payment for lectures from the GSK group of companies, Sanofi Pasteur MSD, Novartis, Crucell/Janssen, Pfizer and payment for manuscript preparation from Novartis. SE, GB MGD, GF, AP, FS report no conflict of interest.

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References

- [1] European Centre for Disease Prevention and Control (ECDC). European monthly measles monitoring (EMMO) Issue 8: 21 February 2012. Available from: Available from: http://ecdc.europa.eu/en/publications/Publications/SUR_EMMO_European-monthly-measles-monitoring-February-2012.pdf [last accessed: 26 April 2016].
- [2] European Centre for Disease Prevention and Control (ECDC). Measles and rubella monitoring January 2016. Available from: Available from: http://reliefweb.int/sites/reliefweb.int/files/resources/2016_issue_1_%20Measles%20rubella%20monitoring_final.pdf [last accessed: 26 April 2016].
- [3] Filia A, Bella A, Rota M, Tavilla A, Magurano F, Baggieri M, et al. Analysis of national measles surveillance data in Italy from October 2010 to December 2011 and priorities for reaching the 2015 measles elimination goal. *Euro Surveill* 2013;18.
- [4] Bonanni P, Ferro A, Guerra R, Iannazzo S, Odone A, Pompa MG, et al. Vaccine coverage in Italy and assessment of the 2012–2014 National Immunization Prevention Plan. *Epidemiol Prev* 2015;39:146–58.
- [5] Ministero della Salute. Coperture vaccinali (%), calcolate sui riepiloghi inviati dalle Regioni e PP.AA. (per singolo antigene). Available from: http://www.salute.gov.it/imgs/C_17_tavole_20_allegati_iitemAllegati_17_fileAllegati_itemFile_0_file.pdf [last accessed: 9 May 2017].
- [6] Bechini A, Boccalini S, Baldo V, Castiglia P, Gallo T, Giuffrida S, et al. Andamento delle coperture vaccinali per varicella ed MPR in alcune regioni Italiane (2009–2014) [Abstract C9.2]. In: 48° Congresso Nazionale Siti; 14–17 Ottobre 2015.
- [7] Ministero della Salute. Piano Nazionale Prevenzione Vaccinale (PNPV) 2012–2014. Available from: http://www.salute.gov.it/imgs/C_17_pubblicazioni_1721_allegato.pdf [last accessed: 17 May 2016].
- [8] Bonanni P, Azzari C, Castiglia P, Chiamenti G, Conforti G, Conversano M, et al. The 2014 lifetime immunization schedule approved by the Italian scientific societies. Italian Society of Hygiene, Preventive Medicine, and Public Health. Italian Society of Pediatrics. Italian Federation of Pediatric Physicians. Italian Federation of General Medical Physicians. Arezzo Service of Legal Medicine. *Epidemiol Prev* 2014;38:131–46.
- [9] Vesikari T, Karvonen A, Bianco V, Van der Wielen M, Miller J. Tetravalent meningococcal serogroups A, C, W-135 and Y conjugate vaccine is well tolerated and immunogenic when co-administered with measles-mumps-rubella-varicella vaccine during the second year of life: an open, randomized controlled trial. *Vaccine* 2011;29:4274–84.
- [10] Klein NP, Shepard J, Bedell L, Odrjij T, Dull P. Immunogenicity and safety of a quadrivalent meningococcal conjugate vaccine administered concomitantly with measles, mumps, rubella, varicella vaccine in healthy toddlers. *Vaccine* 2012;30:3929–36.
- [11] Ferrera G, Cuccia M, Mereu G, Icardi G, Bona G, Esposito S, et al. Booster vaccination of pre-school children with reduced-antigen-content diphtheria-tetanus-acellular pertussis-inactivated poliovirus vaccine co-administered with measles-mumps-rubella-varicella vaccine: a randomized, controlled trial in children primed according to a 2 + 1 schedule in infancy. *Hum Vaccin Immunother* 2012;8:355–62.
- [12] Vesikari T, Karvonen A, Lindblad N, Korhonen T, Lommel P, Willems P, et al. Safety and immunogenicity of a booster dose of the 10-valent pneumococcal nontypeable Haemophilus influenzae protein D conjugate vaccine co-administered with measles-mumps-rubella-varicella vaccine in children aged 12–16 months. *Pediatr Infect Dis J* 2010;29:e47–56.
- [13] Vesikari T, Esposito S, Prymula R, Ypma E, Kohl I, Toneatto D, et al. Immunogenicity and safety of an investigational multicomponent, recombinant, meningococcal serogroup B vaccine (4CMenB) administered concomitantly with routine infant and child vaccinations: results of two randomised trials. *Lancet* 2013;381:825–35.
- [14] Shinefield H, Black S, Thear M, Coury D, Reisinger K, Rothstein E, et al. Safety and immunogenicity of a measles, mumps, rubella and varicella vaccine given with combined Haemophilus influenzae type b conjugate/hepatitis B vaccines and combined diphtheria-tetanus-acellular pertussis vaccines. *Pediatr Infect Dis J* 2006;25:287–92.
- [15] Shinefield H, Black S, Digilio L, Reisinger K, Blatter M, Gress JO, et al. Evaluation of a quadrivalent measles, mumps, rubella and varicella vaccine in healthy children. *Pediatr Infect Dis J* 2005;24:665–9.
- [16] Schuster V, Otto W, Maurer L, Tcherepnine P, Pfletschinger U, Kindler K, et al. Immunogenicity and safety assessments after one and two doses of a refrigerator-stable tetravalent measles-mumps-rubella-varicella vaccine in healthy children during the second year of life. *Pediatr Infect Dis J* 2008;27:724–30.
- [17] Knuf M, Habermehl P, Zepp F, Mannhardt W, Kuttig M, Muttonen P, et al. Immunogenicity and safety of two doses of tetravalent measles-mumps-rubella-varicella vaccine in healthy children. *Pediatr Infect Dis J* 2006;25:12–8.
- [18] Schink T, Holstiege J, Kowalzik F, Zepp F, Garbe E. Risk of febrile convulsions after MMRV vaccination in comparison to MMR or MMR + V vaccination. *Vaccine* 2014;32:645–50.
- [19] Jacobsen SJ, Ackerson BK, Sy LS, Tran TN, Jones TL, Yao JF, et al. Observational safety study of febrile convulsion following first dose MMRV vaccination in a managed care setting. *Vaccine* 2009;27:4656–61.
- [20] Klein NP, Fireman B, Yih WK, Lewis E, Kulldorff M, Ray P, et al. Measles-mumps-rubella-varicella combination vaccine and the risk of febrile seizures. *Pediatrics* 2010;126:e1–8.
- [21] Opri R, Moretti U, Zanoni G. XVIII Relazione sull'attività del "canale verde". dati relativi al; 2014. Available from Available from: <https://servizi.ospedaleuniverona.it/extfiles/internet/93101/attachment/xviii-relazione-canale-verde.pdf> [last accessed: 25 January 2016].
- [22] Cocchio S, Zanoni G, Opri R, Russo F, Baldo V, Collaborative group. A postmarket safety comparison of 2 vaccination strategies for measles, mumps, rubella and varicella in Italy. *Hum Vaccin Immunother* 2016;12:651–4.

- [23] Czajka H, Schuster V, Zepp F, Esposito S, Douha M, Willems P. A combined measles, mumps, rubella and varicella vaccine (Priorix-Tetra): immunogenicity and safety profile. *Vaccine* 2009;27:6504–11.
- [24] Ozaki T, Nishimura N, Kajita Y. Experience with live attenuated varicella vaccine (Oka strain) in healthy Japanese subjects; 10-year survey at pediatric clinic. *Vaccine* 2000;18:2375–80.
- [25] Dennehy PH, Reisinger KS, Blatter MM, Veloudis BA. Immunogenicity of subcutaneous versus intramuscular Oka/Merck varicella vaccination in healthy children. *Pediatrics* 1991;88:604–7.
- [26] Ferrera G, Gajdos V, Thomas S, Tran C, Fiquet A. Safety of a refrigerator-stable varicella vaccine (VARIVAX) in healthy 12- to 15-month-old children: a randomized, double-blind, cross-over study. *Hum Vaccin* 2009;5:455–60.