# Immunogenicity of MenACWY-CRM in Korean Military Recruits: Influence of Tetanus-Diphtheria Toxoid Vaccination on the Vaccine Response to MenACWY-CRM

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The quadrivalent meningococcal conjugate vaccine (MenACWY-CRM) has been introduced for military recruits in Korea since 2012. This study was performed to evaluate the immunogenicity of MenACWY-CRM in Korean military recruits. In addition, the influence of tetanus-diphtheria toxoids (Td) vaccination on the vaccine response to MenACWY-CRM was analyzed. A total of 75 military recruits were enrolled. Among them, 18 received a dose of MenACWY-CRM only (group 1), and 57 received Td three days before MenACWY-CRM immunization (group 2). The immunogenicity of MenACWY-CRM was compared between the two groups. The serum bactericidal activity with baby rabbit complement was measured before and three weeks after immunization against serogroups A, C, W-135, and Y. The geometric mean titers (GMTs) against four serogroups were significantly increased in both groups after immunization. Compared to group 2, group 1 exhibited significantly higher vaccine responses in several aspects: post-immune GMTs against serogroup A and C, seroresponse rates against serogroup A, and a fold increases of titers against serogroup A, C, and Y. MenACWY-CRM was immunogenic against all vaccine-serogroups in Korean military recruits. Vaccine response to MenACWY-CRM was influenced by Td administered three days earlier.

**Key Words:** *Neisseria meningitidis*, serum bactericidal antibody assay, meningococcal vaccine

*Neisseria meningitidis* is a leading global cause of bacterial meningitis and sepsis.[1,2] Permanent disability or death can occur within a few hours of symptom onset, and morbidity and mortality rates are high.[3] An estimated 500000 annual cases of meningococcal disease occur worldwide, with a case fatality rate of 10−15%.[4] The greatest burden of meningococcal disease occurs between the ages of six months and two years, but a second peak occurs among adolescents and young adults.[2] In particular, new military recruits and college freshmen living in dormitories have been found to have a higher risk of disease than other populations of similar age.[2,5,6]

Between 1988 and 1991, the annual incidence of invasive meningococcal diseases in the Korean military was 0.8−3.3 cases per 100000 military recruits, with a 50% mortality rate.[7] Reports of the carrier rate showed that meningococci were isolated from 17.6−21.7% of soldiers, and the majority of the serogroups were B and C.[8] A total of 12 cases of meningococcal disease were reported between August 2000 and July 2001, with an estimated incidence of 2.2 per 100000 soldiers.[9] Among the isolates identified in the patients at that time, the most

prevalent serogroup was C. A recent outbreak at the Nonsan Recruit Training Center in April 2011 has drawn public awareness to the need for meningococcal immunization in the Korean Army. Since 2012, a single dose of quadrivalent meningococcal conjugate vaccine (MenACWY-CRM) has been routinely administered to all new military recruits based on the Military Healthcare Service Act. In addition, tetanus-diphtheria toxoids (Td), measles-mumps-rubella, hepatitis A, and influenza vaccines are also currently administered to new recruits.

Cross reacting material ($CRM_{197}$), a carrier protein in MenACWY-CRM, is a genetically modified non-toxic form of diphtheria toxin included in Td. Due to the immunological cross reactivity between $CRM_{197}$ and diphtheria toxin, concomitant or sequential administration of Td can induce immunologic interference in the anti-meningococcal response.

Therefore, we aimed to evaluate immune responses against the four serogroups included in the MenACWY-CRM in vaccinated military recruits. In addition, we evaluated the impact of prior immunization with Td on the immunogenicity of MenACWY-CRM.

This study was conducted in Korean military recruits who underwent an immunization program. A total of 75 participants were categorized into two groups in 2013. Eighteen participants in group 1 received only a single dose of MenACWY-CRM (Menveo®, Novartis Vaccines and Diagnostics, Cambridge, MA, USA), while 57 participants in group 2 received MenACWY-CRM and Td (SK Td vaccine inj., SK Chemicals Life Science, Seongnam, Korea). In group 2, Td was administered three days before MenACWY-CRM immunization.

Each 0.5 mL dose of MenACWY-CRM contained 10 μg of meningococcal serogroup A oligosaccharide and 5 μg each of oligosaccharides from meningococcal serogroup C, W-135, and Y conjugated to $CRM_{197}$. Each 0.5 mL dose of Td contained ≥2 IU of diphtheria toxoid and ≥20 IU of tetanus toxoid (TT). The vaccines were injected intramuscularly in the deltoid area.

Eligible individuals were healthy male adults who had never received the meningococcal vaccine. Subjects were excluded if they had a history of meningococcal disease, a disease requiring treatment with immunosuppressive drugs, a history of hypersensitivity to vaccines or any vaccine components, or receipt of antibiotics within seven days. Blood samples were collected before and three weeks after MenACWY-CRM vaccination.

Serum bactericidal assay using baby rabbit complement (rSBA) was performed for four meningococcal serogroups (A, C, W-135, and Y) at the Ewha Center for Vaccine Evaluation and Study, as previously described by Maslanka, et al.[10] The target strains used in the rSBA were ATCC13077 for serogroup A, ATCC13102 for serogroup C, NCCP15745 for serogroup W-135, and S-1975 for serogroup Y. ATCC13077 and ATCC13102 were purchased from ATCC (American Type Culture Collection, Rockville, MD, USA), and NCCP15745 was purchased from National Culture Collection for Pathogens (NCCP, Cheon-

gju, Korea). S-1975 was kindly provided by the US Food and Drug Administration (FDA, Silver Spring, MD, USA).

An rSBA titer ≥8 was used for the correlates of protection since it is the putative protective titer that has been shown to predict short-term clinical protection against disease in the UK.[11] The more discriminatory rSBA titer ≥128, which reliably predicts a serum bactericidal assay using human complement (hSBA) titer ≥4,[12] was also used.

Differences in geometric mean titers (GMTs) between pre- and post-vaccine sera were compared using a two-sample, paired t-test after logarithmic transformation. Comparisons between the groups were evaluated with the Mann-Whitney U test for continuous variables and the Pearson $\chi^2$ test or Fisher's exact test for categorical variables. $p$ values less than 0.05 were considered significant. Statistical analysis was performed using the SPSS statistical software (version 18.0; SPSS Inc., Chicago, IL, USA).

The study was conducted in accordance with the Good Clinical Practice Guidelines and the Declaration of Helsinki. This research protocol was approved by the Institutional Review Board of the Korean Armed Force Medical Command (IRB No. AFMC-12-IRB-048). Written informed consent was obtained before enrollment from all participants.

Baseline GMTs were 644, 23, 266, and 22 for serogroups A, C, W-135, and Y, respectively. An increase in GMTs was observed at three weeks post-immunization, with 10.6-, 36.3-, 70.1-, and 146.6-fold increases over baseline with GMTs of 6813; 837; 18642; and 3147 for serogroups A, C, W-135, and Y, respectively (Table 1). The percentage of subjects with rSBA titers ≥8 at baseline was relatively high for serogroups A (97%) and W-135 (84%) and moderate for serogroups C (53%) and Y (45%). After MenACWY-CRM immunization, the rates increased to 100%, 97%, 100%, and 97% for serogroups A, C, W-135, and Y, respectively. The percentage of subjects with rSBA titers ≥128 at baseline was relatively high for serogroup A (93%), moderate for serogroup W-135 (60%), and low for serogroups C (23%) and Y (27%). After MenACWY-CRM immunization, the rates increased to 100%, 85%, 100%, and 96% for serogroups A, C, W-135, and Y, respectively (Table 1).

The percentages of participants with at least a four-fold increase over baseline rSBA titer were 69%, 80%, 89%, and 88% for serogroups A, C, W-135, and Y, respectively (Table 1). When the seroresponse rate was defined as the proportion of subjects who demonstrated seroconversion (change of baseline rSBA titer <8 or <128 to post-immune rSBA titer ≥8 or ≥128, respectively) after immunization or who showed a four-fold increase over baseline rSBA titer, the majority of subjects achieved a seroresponse against serogroups A (69%), C (80%), W-135 (89%), and Y (88%) (Table 1).

There were no differences in baseline GMTs between two groups, with the exception of serogroup Y (Fig. 1A). After immunization, the GMTs for serogroups A (13812) and C (2397) were significantly higher in group 1 compared to group 2 (5451

for serogroup A and 601 for serogroup C) (*p*<0.05). The geometric mean fold increase was higher in group 1 than in group 2 for serogroup A (31 in group 1, 8 in group 2), C (129, 24), W-135 (98, 63), and Y (593, 94) (*p*<0.05 for A, C, and Y) (Fig. 1B).

Reverse cumulative distributions of rSBA titers showed responses to four serogroups at baseline and three-week post-immunization in group 1 and group 2. After immunization, the curves shifted to the right compared to those before immunization in both groups for all four meningococcal serogroups.

The curves for group 1 at three-week post-immunization are farther to the right than those for group 2 for all four meningococcal serogroups, illustrating that the participants in group 1 showed higher rSBA titers than those in group 2 for serogroups A, C, W-135, and Y (Fig. 2).

We showed that MenACWY-CRM induced high seropositive rates, significant increases in the GMTs of rSBA titers, and high seroresponse rates after immunization against serogroups A, C, W-135, and Y. These findings are similar to those of clini-

**Table 1.** Immunogenicity Data Against Meningococcal Serogroups A, C, W-135, and Y before and after Immunization with MenACWY-CRM (n=75)

| | Serogroup A | | Serogroup C | | Serogroup W-135 | | Serogroup Y | |
|---|---|---|---|---|---|---|---|---|
| | Pre-immunization | Post-immunization | Pre-immunization | Post-immunization | Pre-immunization | Post-immunization | Pre-immunization | Post-immunization |
| Geometric mean titer (95% CI) | 644 (398–1043) | 6813 (4180–11107) | 23 (12–45) | 837 (377–1693) | 266 (119–592) | 18642 (9820–35390) | 22 (10–46) | 3147 (1527–6484) |
| Seropositive rate* (95% CI) | | | | | | | | |
| % ≥8 | 97 (91–100) | 100 (95–100) | 53 (41–65) | 97 (91–100) | 84 (74–91) | 100 (95–100) | 45 (34–57) | 97 (91–100) |
| % ≥64 | 97 (91–100) | 100 (95–100) | 33 (23–45) | 93 (85–98) | 81 (71–89) | 100 (95–100) | 31 (21–42) | 96 (89–99) |
| % ≥128 | 93 (85–98) | 100 (95–100) | 23 (14–34) | 85 (75.3–92.4) | 60 (48–71) | 100 (95–100) | 27 (17–38) | 96 (89–99) |
| Geometric mean fold increase (95% CI) | 11 (5–21) | | 36 (16–81) | | 70 (30–165) | | 147 (54–396) | |
| % ≥4-fold increase (95% CI) | 69 (58–80) | | 80 (69–88) | | 89 (80.1–95.3) | | 88 (78.4–94.4) | |
| Seroresponse rate† (95% CI) | | | | | | | | |
| % ≥8 | 69 (58–80) | | 80 (69–88) | | 89 (80–95) | | 88 (78–94) | |
| % ≥64 | 69 (58–80) | | 81 (71–89) | | 89 (80–95) | | 88 (78–94) | |
| % ≥128 | 69 (58–80) | | 83 (72–90) | | 89 (80–95) | | 88 (78–94) | |

MenACWY-CRM, quadrivalent meningococcal conjugate vaccine; rSBA, serum bactericidal assay using baby rabbit complement; CI, confidence interval.
*Seropositive rate was defined as rSBA titer ≥8, ≥64, or ≥128 in participants, †Seroresponse rate was defined as the proportion of subjects who demonstrated seroconversion (change of baseline rSBA titer <8, <64, or <128 to a post-immune rSBA titer ≥8, ≥64, or ≥128) after immunization or who showed a 4-fold increase over baseline in rSBA titer.
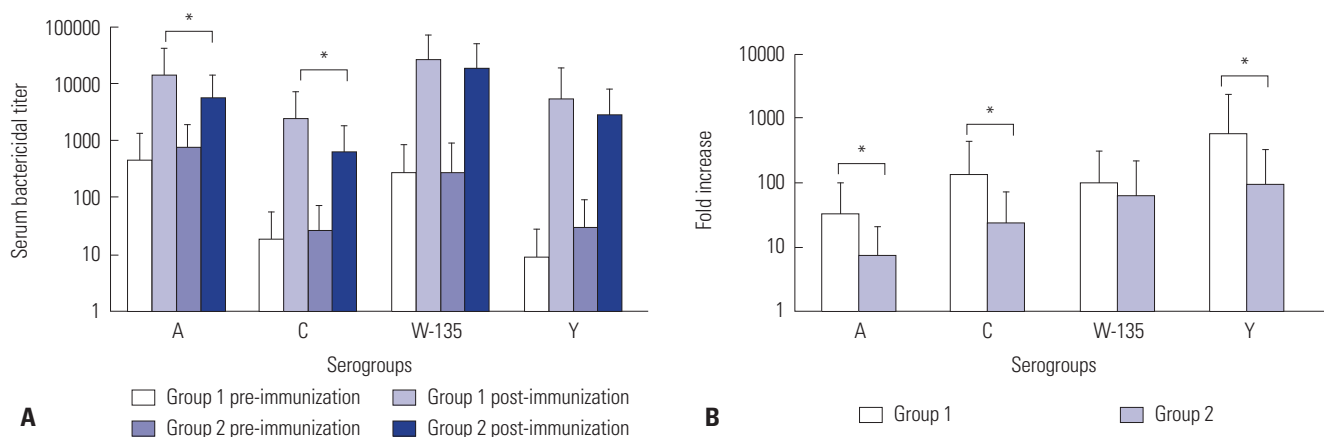


**Fig. 1.** (A) Geometric mean titers and (B) geometric mean fold increase per serogroup and per vaccine group at pre-immunization and at three-week post-immunization. *p*<0.05. Group 1, MenACWY-CRM-only group; Group 2, MenACWY-CRM and tetanus-diphtheria toxoids. MenACWY-CRM, quadrivalent meningococcal conjugate vaccine.
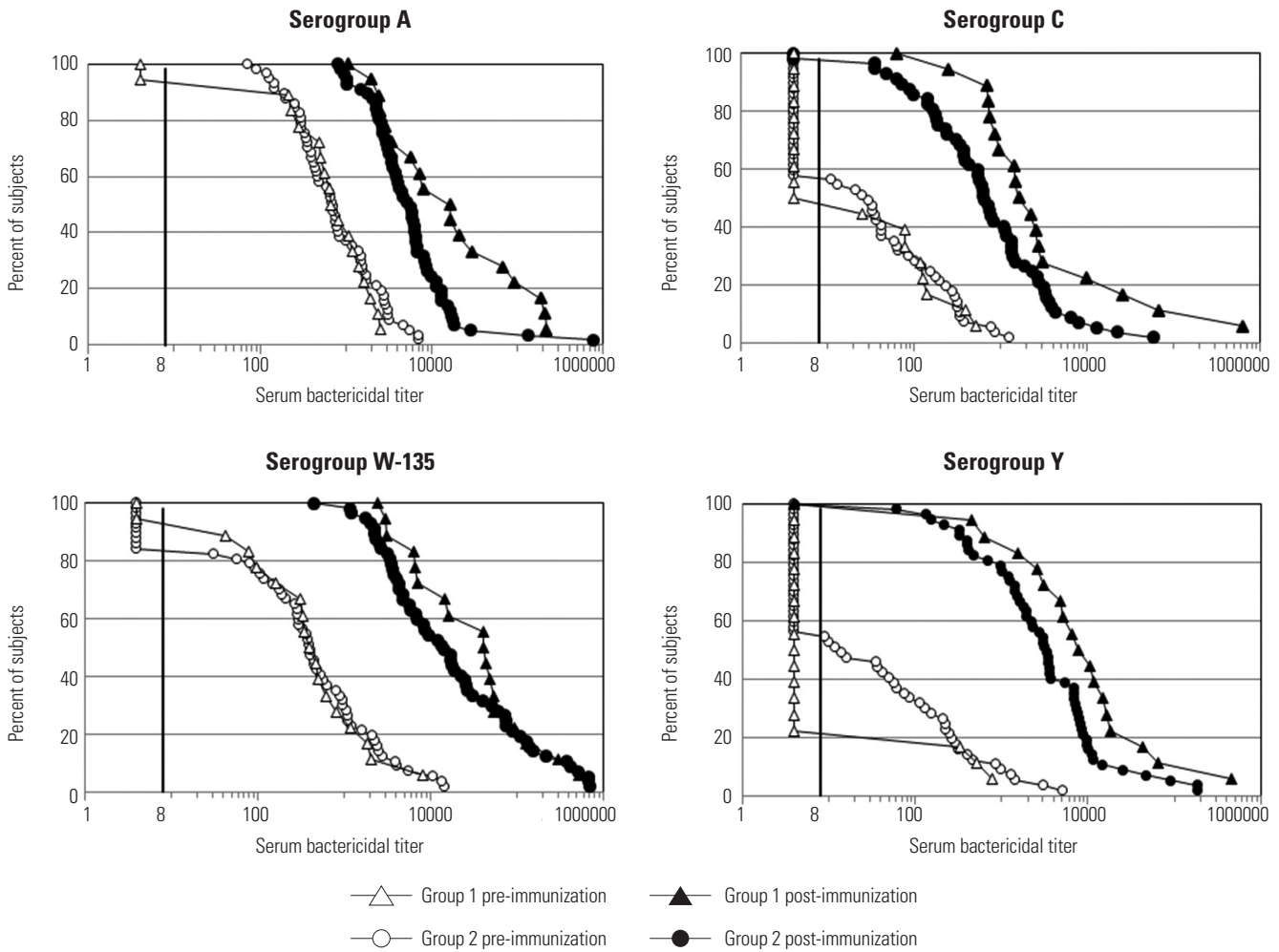
**Fig. 2.** Reverse cumulative distribution curve of rSBA titers against serogroup A, C, W-135, and Y at baseline (Day 0) and at three weeks post-immunization (Day 21) in group 1 (MenACWY-CRM-only group) and group 2 (MenACWY-CRM and tetanus-diphtheria toxoids). Day 0, group 1, open triangle; Day 21, group 1, closed triangle; Day 0, group 2, open circle; Day 21, group 2, closed circle. MenACWY-CRM, quadrivalent meningococcal conjugate vaccine.

cal trials performed in participants aged 11–55 years for the licensure of MenACWY-CRM in Korea and the United States.[13-15] A relatively high baseline immunity for serogroups A and W-135 was shown in our study compared with that of Lee, et al.[14] The low incidence of invasive diseases and carriage rate caused by meningococcal serogroups A and W-135 in Korea might be explained by our results.[8,16-18] These bactericidal antibodies detected in pre-immune sera might be produced by exposure to meningococci colonized in the pharynx or by other bacteria, such as *N. lactamica* or *E. coli* K92, that could induce cross-reactive antibodies.[19,20] The seroprevalence of antibodies against serogroup A or serogroup W-135 meningococci should be performed to confirm this finding and to identify potentially susceptible groups in the population.

An interesting finding in the present study was the effect of prior Td immunization on the immunogenicity of MenACWY-CRM. The post-immune GMTs and geometric mean fold increases in group 1 were significantly higher than those in group 2 in serogroups A and C, and serogroups A, C, and Y, respec-

tively. The influence of prior or concomitant diphtheria toxin immunization on the immunogenicity of MenACWY-CRM was earlier evaluated by several studies with one month intervals of immunization between a Td-containing vaccine and MenACWY-CRM.[21-23] Arguedas, et al.[21] reported that GMTs for serogroups W-135 and Y and the seroresponse rate for serogroup W-135 were lower when MenACWY-CRM was administered one month after Tdap. Nevertheless, they concluded that Tdap and MenACWY-CRM could be administered concomitantly or sequentially without a decreased immune response. Gasparini, et al.[22] showed that the immunogenicity of MenACWY-CRM was not impaired by concomitant administration of Tdap. Similarly, the immune responses to diphtheria and tetanus antigens were non-inferior when Tdap was administered concomitantly with MenACWY-CRM. However, Burrage, et al.[23] observed that the immune response to the meningococcal C vaccine (MCC) conjugated to TT was reduced as a result of prior immunization with a tetanus-containing vaccine, although prior or simultaneous administration of a

diphtheria-containing vaccine did not affect the response to MCC-CRM vaccines. Immunization with a carrier protein alone might induce immune suppression to a hapten linked to the carrier protein. This suppression could occur when the conjugate contains a low ratio of hapten to carrier and when the carrier is overloaded.[24] In adolescents, prior administration of tetanus or diphtheria vaccine has been shown to reduce the immune response to meningococcal vaccine conjugated to diphtheria or TT.[24] However, to the best of our knowledge, there have been no trials with a shorter immunization interval (less than one month) between meningococcal vaccine and a diphtheria-containing vaccine. Further evaluation is needed to clarify our results that prior Td immunization reduced the immune response to a certain serogroup in MenACWY-CRM.

One limitation of the present study is the small sample size for clarifying the differences between the two vaccine groups. Because this study was performed according to an immunization program on military recruits, we could not enroll subjects who did not need the Td vaccine into group 1.

Baby rabbit complement was used as a source of complement for the serum bactericidal assay. The proven correlate of a protective titer against meningococcal disease is a serum bactericidal titer of 1:4 or higher according to the Goldschneider, et al.[12] assay using human complement. However, one limitation of using human complement is that it is difficult to obtain normal human serum that lacks intrinsic bactericidal antibodies. Instead, an rSBA titer ≥8 has been used as a correlate of protection since it is the putative protective titer that has been shown to predict short-term clinical protection against disease in the UK.[11,25,26] A more discriminatory rSBA titer ≥128, which reliably predicts a hSBA titer ≥4, has also been used to analyze immunogenicity in many studies.[25] Therefore, our data are sufficient to demonstrate the immunogenicity of MenACWY-CRM in this population.

In conclusion, MenACWY-CRM elicited a good immune response to all vaccine serogroups in Korean military recruits. Immune response to MenACWY-CRM was affected by Td administered three days earlier. Because of the limited number of subjects enrolled, further evaluation is needed to confirm this finding and to optimize the immunization schedule.

## ACKNOWLEDGEMENTS

## REFERENCES

1. Harrison LH, Pass MA, Mendelsohn AB, Egri M, Rosenstein NE, Bustamante A, et al. Invasive meningococcal disease in adolescents and young adults. JAMA 2001;286:694-9.
2. Rosenstein NE, Perkins BA, Stephens DS, Popovic T, Hughes JM. Meningococcal disease. N Engl J Med 2001;344:1378-88.
3. Thompson MJ, Ninis N, Perera R, Mayon-White R, Phillips C, Bailey L, et al. Clinical recognition of meningococcal disease in children and adolescents. Lancet 2006;367:397-403.
4. Agrawal S, Nadel S. Acute bacterial meningitis in infants and children: epidemiology and management. Paediatr Drugs 2011;13:385-400.
5. Broderick MP, Faix DJ, Hansen CJ, Blair PJ. Trends in meningococcal disease in the United States military, 1971-2010. Emerg Infect Dis 2012;18:1430-7.
6. Meningococcal disease and college students. Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 2000;49:13-20.
7. Park HS, Chun YI. Vaccination effect on pharyngeal carrier rate of Neisseria meningitidis and its serogroups in Korean Army recruits. J Korean Mil Med Assoc 1992;23:105-15.
8. Hwang IU, Lee HK, Seo MY, Kim JP, Seo YB, Bang YJ. The changes of Meningococcal carriage rate and the serogroup in Korean army recruits. J Korean Mil Med Assoc 2010;41:188-99.
9. Lee SO, Ryu SH, Park SJ, Ryu J, Woo JH, Kim YS. Meningococcal disease in the republic of Korea army: incidence and serogroups determined by PCR. J Korean Med Sci 2003;18:163-6.
10. Maslanka SE, Gheesling LL, Libutti DE, Donaldson KB, Harakeh HS, Dykes JK, et al. Standardization and a multilaboratory comparison of Neisseria meningitidis serogroup A and C serum bactericidal assays. The Multilaboratory Study Group. Clin Diagn Lab Immunol 1997;4:156-67.
11. Andrews N, Borrow R, Miller E. Validation of serological correlate of protection for meningococcal C conjugate vaccine by using efficacy estimates from postlicensure surveillance in England. Clin Diagn Lab Immunol 2003;10:780-6.
12. Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus. I. The role of humoral antibodies. J Exp Med 1969;129:1307-26.
13. Jackson LA, Baxter R, Reisinger K, Karsten A, Shah J, Bedell L, et al. Phase III comparison of an investigational quadrivalent meningococcal conjugate vaccine with the licensed meningococcal ACWY conjugate vaccine in adolescents. Clin Infect Dis 2009;49:e1-10.
14. Lee HJ, Chung MH, Kim WJ, Hong YJ, Choi KM, Lee J, et al. Immunogenicity and safety of a novel quadrivalent meningococcal conjugate vaccine (MenACWY-CRM) in healthy Korean adolescents and adults. Int J Infect Dis 2014;28:204-10.
15. Reisinger KS, Baxter R, Block SL, Shah J, Bedell L, Dull PM. Quadrivalent meningococcal vaccination of adults: phase III comparison of an investigational conjugate vaccine, MenACWY-CRM, with the licensed vaccine, Menactra. Clin Vaccine Immunol 2009;16:1810-5.
16. Kim SA, Kim DW, Dong BQ, Kim JS, Anh DD, Kilgore PE. An expanded age range for meningococcal meningitis: molecular diagnostic evidence from population-based surveillance in Asia. BMC Infect Dis 2012;12:310.
17. Bae SM, Kang YH. Serological and genetic characterization of meningococcal isolates in Korea. Jpn J Infect Dis 2008;61:434-7.
18. Heo JY, Bae SM, Cheong HJ, Kim WJ, Kim MY, Na W, et al. Impact of quadrivalent meningococcal conjugate vaccine on carried meningococci in Korean Military trainee. J Korean Mil Med Assoc 2014;45:33-42.
19. Glode MP, Robbins JB, Liu TY, Gotschlich EC, Orskov I, Orskov F. Cross-antigenicity and immunogenicity between capsular polysaccharides of group C Neisseria meningitidis and of Escherichia coli K92. J Infect Dis 1977;135:94-104.
20. Gold R, Goldschneider I, Lepow ML, Draper TF, Randolph M. Carriage of Neisseria meningitidis and Neisseria lactamica in infants and children. J Infect Dis 1978;137:112-21.

21. Arguedas A, Soley C, Loaiza C, Rincon G, Guevara S, Perez A, et al. Safety and immunogenicity of one dose of MenACWY-CRM, an investigational quadrivalent meningococcal glycoconjugate vaccine, when administered to adolescents concomitantly or sequentially with Tdap and HPV vaccines. Vaccine 2010;28:3171-9.

22. Gasparini R, Conversano M, Bona G, Gabutti G, Anemona A, Dull PM, et al. Randomized trial on the safety, tolerability, and immunogenicity of MenACWY-CRM, an investigational quadrivalent meningococcal glycoconjugate vaccine, administered concomitantly with a combined tetanus, reduced diphtheria, and acellular pertussis vaccine in adolescents and young adults. Clin Vaccine Immunol 2010;17:537-44.

23. Burrage M, Robinson A, Borrow R, Andrews N, Southern J, Findlow J, et al. Effect of vaccination with carrier protein on response to meningococcal C conjugate vaccines and value of different immunoassays as predictors of protection. Infect Immun 2002;70:4946-54.

24. Pobre K, Tashani M, Ridda I, Rashid H, Wong M, Booy R. Carrier priming or suppression: understanding carrier priming enhancement of anti-polysaccharide antibody response to conjugate vaccines. Vaccine 2014;32:1423-30.

25. Borrow R, Andrews N, Goldblatt D, Miller E. Serological basis for use of meningococcal serogroup C conjugate vaccines in the United Kingdom: reevaluation of correlates of protection. Infect Immun 2001;69:1568-73.

26. Borrow R, Balmer P, Miller E. Meningococcal surrogates of protection--serum bactericidal antibody activity. Vaccine 2005;23:2222-7.