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Original Article

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Body Weight is Inversely Associated with Anti-SARS-CoV-2 Antibody Levels after BNT162b2 mRNA Vaccination in Young and Middle Aged Adults

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ABSTRACT

Background: This study aimed to determine factors affecting serum levels of anti-severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies 2 months after coronavirus disease 2019 (COVID-19) vaccination in young and middle aged healthy adults.
Materials and Methods: Healthcare workers who have no history of SARS-CoV-2 infection, were enrolled at 2 months after second shot of BNT162b2 mRNA COVID-19 vaccine. Antibody immunoglobulin G against the spike protein subunit of SARS-CoV-2 was semi-quantitatively measured using 4 commercial enzyme-linked immunosorbent assay kits. Factors affecting anti-SARS-CoV-2 antibodies levels were investigated.
Results: Fifty-one persons (22 - 54 years, male sex; 19.6%) were enrolled and all participants acquired anti-SARS-CoV-2 antibodies in four diagnostic kits. Anti-SARS-CoV-2 antibodies were strongly correlated between diagnostic kits; SG Medical and Genscript (r = 0.942), SG Medical and HB Healthcare (r = 0.903), and HB Healthcare and Genscript (r = 0.868). We investigated factors affecting antibody level using SG medical kit. The median inhibition was 93.1%, and 84.0% of participants showed >90.0% inhibition. Systemic adverse event severity had no association with the anti-SARS-CoV-2 antibodies level. Antibody level was inversely correlated with weight (-0.312, P = 0.027), body mass index (BMI) (r = -0.303, P = 0.032), and body surface area (r = -0.285, P = 0.044). In multivariate analysis, the upper 50% of anti-SARS-CoV-2 antibodies (≥93.1%) was inversely associated with weight (odds ratio [OR]: 0.19; 95% confidence interval [CI]: 0.04 - 0.83 in weight ≥55kg) and BMI (OR: 0.12; 95% CI: 0.03 - 0.61 in BMI ≥22 kg/m^2).
Conclusion: Anti-SARS-CoV-2 antibody was inversely correlated with weight and BMI, which may be used as a marker to predict immune response of BNT162b2 mRNA vaccination in young and middle aged adults.

Trial Registration: ClinicalTrials.gov Identifier: NCT05083026

Keywords: Anti-SARS-CoV-2 antibodies; Vaccination; COVID-19

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Conflict of Interest
No conflict of interest.

Author Contributions
Conceptualization: SYN. Data curation: SYN, DKJ. Formal analysis: SYN. Funding acquisition: SYN. Investigation: SY Nam, SWJ, DKJ, SJH. Methodology: SYN, DKJ. Project administration: SYN. Resources: SY Nam, SWJ, SJH. Software: SYN. Supervision: SYN, SWJ, SJH. Validation: SYN. Visualization: SYN. Writing - original draft: SYN, DKJ. Writing - review & editing: SYN, SWJ, SJH.

INTRODUCTION

The wide spread occurrence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has induced a pandemic and rapid development of vaccines. SARS-CoV-2 is known to infect individuals by binding to the angiotensin-converting enzyme 2 (ACE2) receptor of the host cell via the receptor-binding domain (RBD) of the spike (S) protein [1]. In Korea, coronavirus disease 2019 (COVID-19) vaccine administration began on February 26, 2021. Currently, Korea has authorized two mRNA vaccines, BNT162b2 by Pfizer-BioNTech (Pfizer, New York, NY, USA) and mRNA-1273 (Moderna, Norwood, MA, USA) two adenoviral vector-based vaccines ChAdOx1-S by AstraZeneca-Oxford (SK bioscience, Andog-si, Gyeongsangbuk-do, Korea), and COVID-19 Vaccine Janssen by Janssen Biologics B.V. (Baltimore, Marylan, USA) and Novavax vaccine (Novavax, Gaithersburg, Maryland, USA). All vaccines target the S protein of SARS-CoV-2 [2], and all have induced anti-S IgG antibodies with neutralizing activity against the first pandemic of the SARS-CoV-2 Wuhan Hu-1 variant [3, 4].

Randomized clinical trials demonstrated the efficacy of COVID-19 vaccines, [5, 6] and recent research suggests that neutralizing antibody levels after vaccination are highly predictive of immune protection from symptomatic SARS-CoV-2 infection [7-9]. The efficacy looks to be related with individual variance in the level of anti-SARS-CoV-2 antibody after vaccination. Recently, we suggested demographic factors affecting to anti-SARS-CoV-2 antibody 6 months after vaccination [10]. Studies from Korea and Japan suggest that systemic adverse events have no association with immunogenicity after vaccination [11, 12]. Healthcare workers are one of high-risk groups of SARS-CoV-2 exposure [13] and they were vaccinated early. Here, we measured anti-SARS-CoV-2 antibodies using four different commercial diagnostic kits among a serum sample cohort of 51 Korean healthcare workers who received two doses of SARS-CoV-2 BNT162b2 mRNA vaccine (Pfizer, USA). We investigated the associated factors affecting anti-SARS-CoV-2 antibody levels at 2 months after 2 doses of mRNA vaccines.

MATERIALS AND METHODS

1. Study design and enrollment

This observational study included healthy healthcare workers who received a SARS-CoV-2 BNT162b2 mRNA vaccine (Pfizer, USA) in Kyungpook National University Chilgok Hospital, Korea. Participants received two injections 3 weeks apart (first dose: March 17th - 20th and second dose: April 7th – 10th). No participant had a history of SARS-CoV-2 infection. The vaccination center provided two tablets of Tylenol® (Janssen Korea, Suwon, Gyeonggi-do, Korea) to all persons on the day of vaccination for on-demand use. Serum samples were collected 2 months after the second BNT162b2 (Pfizer, USA) injection. Participants completed demographic questionnaires [age (birth year and month), sex, work place, weight, height, smoking status, alcohol consumption, presence of chronic disease, and use of any medication] and questionnaires for adverse events and use of antipyretics. Body mass index (BMI) was calculated as weight/height^2 (kg/m^2). Body surface area (BSA) was calculated with the Mosteller formula (m^2) [14]:

$$BSA = \frac{\sqrt{W \times H}}{60} = 0.016667 \times W^{0.5} \times H^{0.5}$$

where W (weight) was expressed in kilograms and H (height) in centimeters.

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2. Ethics statement

The Kyungpook National University Hospital Chilgok Institutional Review Board approved the study (KNUCH 2021-05-001-001), and the participants signed written informed consents. This study is registered at *ClinicalTrials.gov* under registration (NCT05083026).

3. Adverse events assessment

Local (injection site pain, swelling, redness) and systemic [fever, chills, headache, fatigue, muscle pain (myalgia), joint pain (arthralgia), nausea, lymphedema, general edema, sensory neuropathy, motor weakness, etc.] adverse events were assessed using the U.S. Food and Drug Administration guidelines. Grade 0 indicates no adverse events in all categories. Adverse events were graded as follows: Grade 1, did not interfere with activity; Grade 2, interfered with activity; Grade 3, prevented daily activity; and Grade 4, required an emergency department visit or hospitalization. Fever was defined as Grade 1 (37.5 and 37.9), Grade 2 (38 - 38.9), Grade 3 (39 - 40) and Grade 4 (>40). Any systemic event grade was defined according to highest grade of any systemic event.

4. Measurement of anti-SARS-CoV-2 antibodies

Serum collected using BD Vacutainer SST™ Advance tubes (BD, Plymouth, Devon, UK) was centrifuged for 21 min at 2347 rpm after clotting for 30 min. Each aliquot was stored at -80°C. Neutralizing antibody immunoglobulin G (IgG) against S protein subunit (S1) of SARS-CoV-2 was semi-quantitatively measured using four different enzyme-linked immunosorbent assay (ELISA) kits according to manufacturer's recommendations; namely, R-FIND SARS-CoV-2 Neutralizing Antibody ELISA (E1008, SG Medical, Seoul, Korea); SARS-CoV-2 Surrogate Virus Neutralization Test (sVNT) Kit (Genscript, Nanjing, Jiangsu, China); HBelisa™ SARS-CoV-2 Neutralizing Antibody Detection Kit (HB-EK001, HB Healthcare, Cheongju, Chungcheonbuk-do, Korea); and SGT SARS-CoV-2 *in vitro* Neutralizing Antibody Test (IVnAT) (Sugentech, Cheongju, Chungcheonbuk-do, Korea). All ELISA kits measured neutralization antibodies against SARSCoV-2 that block the interaction between the RBD of the viral spike glycoprotein with the ACE2 cell surface receptor in serum or plasma (**Supplementary Table 1**). The target of the HB Healthcare kit is ACE2 and of the three other kits is the RBD.

5. Definition of inhibition (calculation equation) and cut-off value in each diagnostic kit

Inhibition percent or ratio was calculated using optical density (OD) according to the manufactures recommendation as follow:

SG medical, Genscript, and Sugentech;

$$\text{Signal Inhibition} = [1 - (\text{OD of Sample} / \text{OD of Negative control})] \times 100$$

HB Healthcare;

$$\text{Ratio} = \text{OD of Sample} / \text{OD of Calibrator [Company's definition]}$$

$$\text{Signal Inhibition} = [1 - (\text{OD of Sample} / \text{OD of Calibrator})] \times 100 \text{ [Researcher's definition]}$$

6. Statistical analyses

Data are presented as mean ± standard deviation (SD) or number (percent). Correlation of anti-SARS-CoV-2 antibodies level (inhibition percent) between each diagnostic kit was measured using Spearman's correlation. Correlation of demographic factors and systemic adverse event grade with anti-SARS-CoV-2 antibodies level was also measured using Spearman's correlation.

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Weight, BMI, and BSA had no linear association. We created fit curve (smooth spline with 4 knots) for weight, BMI, and BSA (**Supplementary Fig. 1**). We classified them into 2 or 3 categories based on fit curve and distribution of participants. Age (<39, ≥39 years), weight (<55, 55 - 66, ≥67 kg), BMI (<22, ≥22 kg/m^2), and BSA (<1.7, ≥1.7 m^2) were categorized for further analysis. Independent t-test or analysis of variance (ANOVA) was used to investigate the difference of anti-SARS-CoV-2 antibodies levels according to sex, systemic adverse event grade, Tylenol user after vaccination, and categorized data of age, weight, BMI, and BSA.

For further analysis, anti-SARS-CoV-2 antibodies were categorized into upper 50% and lower 50% (cut-off is median: 93.1%). The difference of epidemiologic factors according to binary anti-SARS-CoV-2 antibodies was tested with a *t*-test for continuous variables and chi-square or Fisher's exact test for categorical variables. In multivariate analysis using logistic regression with odds ratio (OR) and 95% confidence interval (CI), binary anti-SARS-CoV-2 antibodies were set as dependent variables and BMI (model I), BSA (model II), or weight (model III) were set as independent variables. Additional covariates were age, sex, and Tylenol® user. Scatter plots of anti-SARS-CoV-2 antibodies were created with GraphPad Prism software, Version 9.0 (GraphPad Software Inc, California, CA, USA). Simple linear regression and interpolation analysis was performed using GraphPad. Statistical analyses were performed using STATA software (version 15; College Station, TX, USA). All statistical tests were two-sided, and P <0.05 was considered statistically significant.

RESULTS

1. Study population

Fifty-one healthy healthcare workers who received a SARS-CoV-2 vaccine were enrolled [mean age: 34.9 years, age range: 22 – 54 years, 10 male sex (19.6%)]. Weight range was 42 - 92 kg (mean, 58.3 kg) and BMI range was 17 - 29 kg/m^2 (mean, 22.5 kg/m^2). Most persons (n = 50) received 0.3 mL (30 μg per dose) in the first and second doses, and one person received 0.2 mL (20 μg per dose) in the first and second doses (**Fig. 1A**). The one person who received 0.2 mL of BNT162b2 (Pfizer, USA) was excluded for analysis.

2. Local and systemic adverse events after SARS-CoV-2 vaccination

Among the 50 participants who received the second injection of BNT162b2 (Pfizer, USA), 43 (86.0%) reported local adverse events and 40 (80.0%) reported systemic adverse events. The most common local adverse event was local pain (n = 42, 84.0%), followed by redness (n = 24, 48.0%) and local edema (n = 23, 46.0%). The most common systemic adverse event was myalgia (n = 30, 60.0%), followed by fever (n = 21, 42.0%), headache (n = 16, 32.0%), chill (n = 14, 28.0%), general weakness (n = 12, 24.0%), nausea (n = 6, 12.0%), and others (lymphedema: n = 1; arthralgia: n = 1; dizziness: n = 1; urticarial: n = 1; diarrhea and abdominal pain: n = 2). Most individual systemic adverse events were grades 1 - 2 (**Supplementary Fig. 2**) and lasted ≤3 days in 98% of cases. We categorized the adverse event grades into 0, 1, and 2 - 3. Twenty-eight persons (56.0%) took acetaminophen (Tylenol®), and two persons received additional NSAIDs.

3. Semi-quantitative levels of anti-SARS-CoV-2 antibody in four different ELISA kits

All participants (n = 51) acquired neutralizing antibody at 2 months after the second injection of COVID-19 mRNA vaccine, BNT162b2 (Pfizer, USA), in four commercial ELISA kits (**Fig. 1B**). Inhibition strength was variable in each person in the SG Medical (80.3 - 94.3%), Genscript

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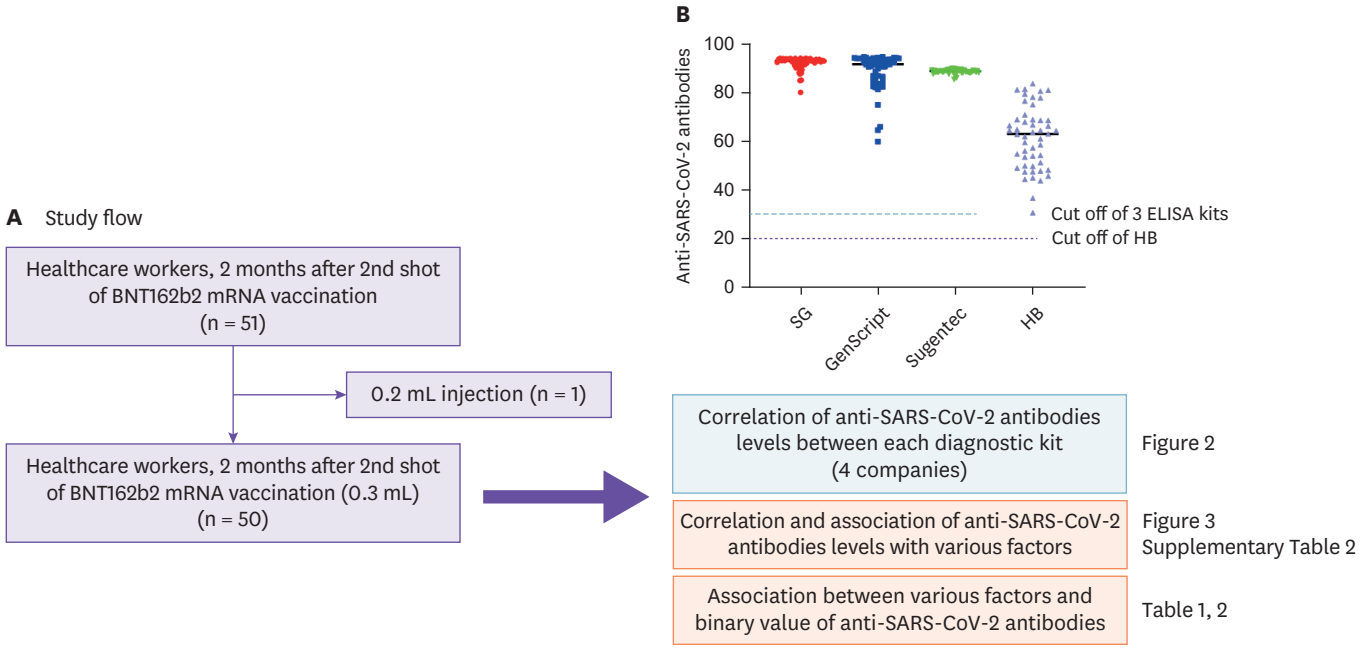

Figure 1. Study flow and outcome.
(A) Study flow. A total of 51 healthcare workers were enrolled and anti-SARS-CoV-2 antibodies was measured 2 month after 2nd shot of BNT162b2 mRNA vaccination (Pfizer, USA). (B) Overview of anti-SARS-CoV-2 antibodies in four commercial ELISA (enzyme-linked immunosorbent assay) kits. Blue dot line indicates cut off values for positive antibody in SG medical, GenScript, and Sugentec. Purple dot line indicates cut off values for positive antibody in HB healthcare.
HB, HB healthcare kit; SG, SG medical kit; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2.

(60.2 - 94.9%), and HB Healthcare (31.3 - 83.9%) kits, whereas the inhibition range had a slight individual variance in the Sugentech kit (85.6 - 90.1%) (**Fig. 1B** and **Supplementary Table 1**). Anti-SARS-CoV-2 antibodies levels were strongly correlated between diagnostic kits: SG Medical and Genscript (r = 0.942), SG Medical and HB Healthcare (r = 0.903), and HB Healthcare and Genscript (r = 0.868) (**Fig. 2**).

4. Correlation between anti-SARS-CoV-2 antibody levels and demographic factors

We measured the correlation between serum level of anti-SARS-CoV-2 antibody measured by 4 different diagnostic kits and demographic factors (**Supplementary Table 2**). The anti-SARS-CoV-2 antibodies level measured with the SG Medical kit was inversely correlated with weight (r = -0.312, P = 0.027), BMI (r = -0.303, P = 0.032), BSA (r = -0.285, P = 0.044), and weight/height (r = -0.318, P = 0.024) (**Fig. 3A**, **3B**). In the HB Healthcare kit, anti-SARS-CoV-2 antibodies level was inversely correlated with weight (r = -0.304, P = 0.032), BMI (r = -0.293, P = 0.039), BSA (r = -0.274, P = 0.054), and weight/height (r = -0.299, P = 0.035) (**Fig. 3A**). Overall systemic adverse event grade had no correlation with anti-SARS-CoV-2 antibodies levels in all kits.

5. Level of anti-SARS-CoV-2 antibodies by categorical variables

Younger age (<39 years), lower body weight (<55 kg), lower BSA (<1.7 m^2), and lower weight/height ratio (<0.35) were associated with higher levels of anti-SARS-CoV-2 antibodies (**Fig. 3C**, **Supplementary Table 3**). Women and Tylenol® user showed higher but statistically insignificant levels of anti-SARS-CoV-2 antibodies (P = 0.066). These features were similar with the Genscript and HB Healthcare kits. A lower BSA (<1.7 m^2) and use of Tylenol® correlated with a

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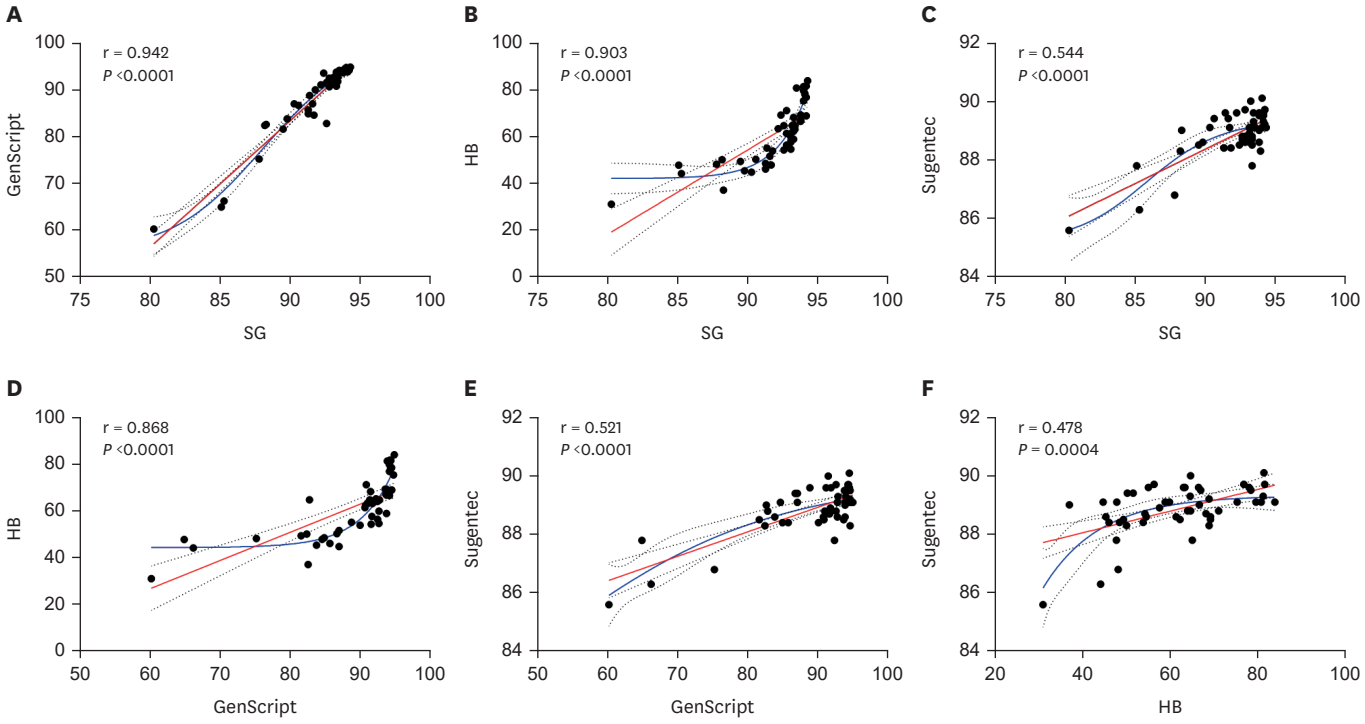

Figure 2. Correlation of anti-SARS-CoV-2 antibodies level with each detection kit.
(A) Correlation of anti-SARS-CoV-2 antibodies between SG medical and Genscript. (B) Correlation of anti-SARS-CoV-2 antibodies between SG medical and HB healthcare. (C) Correlation of anti-SARS-CoV-2 antibodies between SG medical and Sugentech. (D) Correlation of anti-SARS-CoV-2 antibodies between Genscript and HB healthcare. (E) Correlation of anti-SARS-CoV-2 antibodies between Genscript and Sugentech. (F) Correlation of anti-SARS-CoV-2 antibodies between HB healthcare and Sugentech.
Scatter plots of distribution of anti-SARS-CoV-2 antibodies were created with GraphPad Prism software, version 9.0 (GraphPad Software). Red solid lines indicate simple linear regression and blue solid lines indicate interpolation analysis. Dot line indicates 95% confidence interval.
HB, HB healthcare kit; SG, SG medical kit; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2.

higher level of anti-SARS-CoV-2 antibodies in the Sugentech kit. Anti-SARS-CoV-2 antibodies showed no difference according to systemic adverse event grade in all four ELISA kits.

Differences of anti-SARS-CoV-2 antibodies according to age and sex may be explained by weight differences according to age and sex (**Supplementary Table 4**). Younger age (<39 years) showed lower body weight (mean weight: 55.9 *vs.* 61.6 kg, $P = 0.042$), and women had lower body weight (55.1 *vs.* 70.9 kg, $P < 0.001$) than men.

6. Association between demographic factors and binary anti-SARS-CoV-2 antibodies

Among 50 participants, 42 (84.0%) showed >90.0% inhibition. We categorized anti-SARS-CoV-2 antibodies into upper and lower 50% using the SG medical kit (cut off is median; 93.1%). The upper 50.0% of anti-SARS-CoV-2 antibodies showed lower values of BMI, body weight, BSA, and weight/height (**Table 1**). Categorized weight, BMI, BSA, and weight/height were also significantly different according to binary anti-SARS-CoV-2 antibodies (**Table 1**). Sex, age, smoking status, chronic disease, and use of any medication had no association with binary anti-SARS-CoV-2 antibodies.

7. Odds ratio for upper 50% of anti-SARS-CoV-2 antibodies (adjusted analysis)

Adjusted analysis of weight and BSA as continuous variables showed a significant negative association with binary anti-SARS-CoV-2 antibodies. Categorized weight >55 kg (adjusted OR

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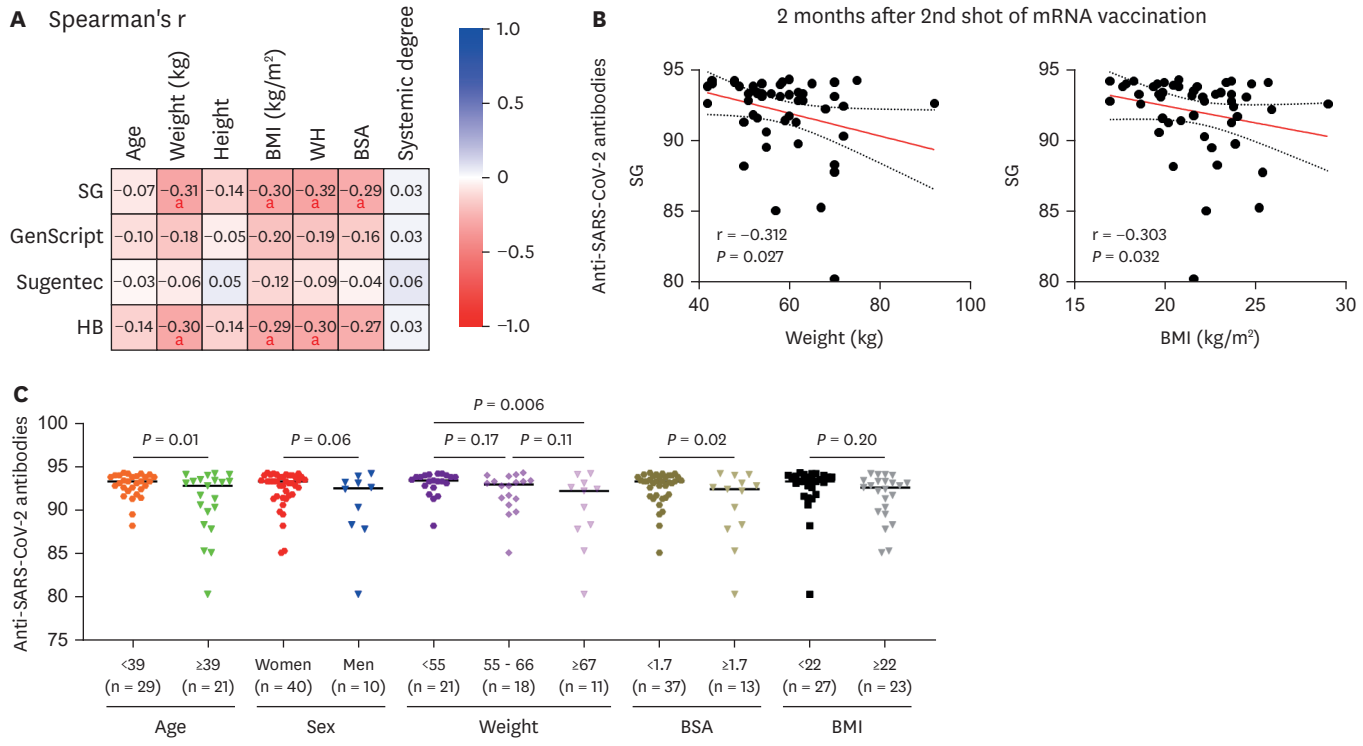

Figure 3. Correlation of anti-SARS-CoV-2 antibodies levels with various factors.
(A) Correlation coefficient (rho) between factors and anti-SARS-CoV-2 antibodies levels in each diagnostic kit. [a]Refers to *P*-value <0.05. Exact correlation coefficient and *P*-values were provided in **Supplementary Table 2**.
(B) Scatter graph between anti-SARS-CoV-2 antibodies and factors in SG medical kit. Red solid lines indicate simple linear regression and dot line indicates 95% confidence interval.
(C) Anti-SARS-CoV-2 antibodies levels measured with SG medical kit according to categorical variables.
AE, adverse event; BMI, body mass index; BSA, body surface area; HB, HB healthcare kit; SG, SG medical kit; WH, weight/height ratio; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2.

[aOR]: 0.19; 95% CI: 0.04 – 0.83) and BMI >22 kg/m² (aOR: 0.12; 95% CI: 0.03 – 0.61) had a negative association with the upper 50.0% of anti-SARS-CoV-2 antibodies (**Table 2**). Use of Tylenol® was associated with higher anti-SARS-CoV-2 antibodies in various models (aOR: 3.93 - 4.27). Age and sex had no association with binary anti-SARS-CoV-2 antibodies levels in any model of adjusted analysis.

8. Sub analysis (women)

Women were sub-analyzed because 80% of participants were women. The BSA cutoff value was determined by the median for women (<1.57 m² and ≥1.57 m²). The differences of variables were tested according to binary anti-SARS-CoV-2 antibodies (**Supplementary Table 5**). In adjusted analysis, weight >55 kg (aOR: 0.20; 95% CI: 0.05 - 0.87), BMI >22 kg/m² (aOR: 0.17; 95% CI: 0.03 - 0.93), and BSA >1.57 m² (aOR: 0.21; 95% CI: 0.05 - 0.97) had negative associations with the upper 50% of anti-SARS-CoV-2 antibodies (**Table 2**).

DISCUSSION

Anti-SARS-CoV-2-specific antibody levels 2 months after COVID-19 vaccination is inversely correlated with body weight and BMI in young and middle aged healthy adults in various analysis. This association was similar in women sub-analysis. There are a strong correlation

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Table 1. Association between variables and binary value of anti-SARS-CoV-2 antibodies

Characteristics	Lower 50% (<93.1%) (n = 26)	Upper 50% (≥93.1%) (n = 24)	*P*-value
Male sex, no (%)	7 (27)	3 (13)	0.20
Current drinker, no (%)	22 (85)	18 (75)	0.40
User of Tylenol, no (%)	12 (46)	16 (67)	0.14
Age, mean (SD)	34.3 (8.6)	35.1 (10.3)	0.76
BMI, mean (SD)	22.4 (2.6)	20.9 (2.3)	0.03
Weight, mean (SD)	61.4 (10.2)	54.9 (8.3)	0.02
BSA, mean (SD)	1.67 (0.17)	1.57 (0.14)	0.02
Weight/height (Kg/cm), mean (SD)	0.37 (0.05)	0.34 (0.32)	0.02
Weight × height (Kg × m) , mean (SD)	101.9 (21.3)	89.2 (16.3)	0.02
Weight category, no (%)			0.03
<55 (n = 21)	7 (27)	14 (58)	
55 - 66 (n = 18)	10 (39)	8 (32)	
≥67 (n = 11)	9 (34)	2 (8)	
BMI category, no (%)			0.02
<22 (n = 27)	10 (39)	17 (71)	
≥22 (n = 23)	16 (61)	7 (29)	
Weight/height (kg/m), no (%)			0.03
<0.35 (n = 23)	8 (31)	15 (62)	
≥0.35 (n = 27)	18 (69)	9 (38)	
BSA category, no (%)			0.04
<1.7 (n = 37)	16 (62)	21 (88)	
≥1.7 (n = 13)	10 (38)	3 (12)	

Anti-SARS-CoV-2 antibodies were measured using SG medical. BSA cut off was determined by median.
P-values are derived from chi-square test or t-test.
SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; BMI, body mass index; SD, standard deviation; BSA, body surface area.

Table 2. Odd ratio for upper 50% of anti-SARS-CoV-2 antibodies (adjusted analysis)

Continuous variable	Total population					Women		
	OR (95% CI)	*P*-value	Categorical variable	OR (95% CI)	*P*-value	Categorical variable	OR (95% CI)	*P*-value
Model 1			Model I			Model I		
Women sex	2.74 (0.5 - 15.11)	0.25	Women sex	3.77 (0.60 - 23.60)	0.16			
Age	1.06 (0.98 - 1.14)	0.14	Age	1.1 (1 - 1.21)	0.05	Age	1.08 (0.98 - 1.19)	0.09
BMI	0.76 (0.57 - 1.01)	0.06	BMI ≥22kg/m^2	0.12 (0.03 - 0.61)	0.01	BMI ≥22kg/m^2	0.17 (0.03 - 0.93)	0.04
User of Tylenol	2.76 (0.77 - 9.9)	0.12	User of Tylenol®	4.27 (1.07 - 17.05)	0.04	User of Tylenol	2.72 (0.65 - 11.39)	0.17
Model II			Model II			Model II		
Women sex	0.74 (0.08 - 6.79)	0.79	Women sex	1.44 (0.18 - 11.52)	0.73			
Age	1.06 (0.98 - 1.15)	0.12	Age	1.04 (0.97 - 1.12)	0.26	Age	1.07 (0.98 - 1.16)	0.15
BSA	0.001 (0.0 - 0.45)	0.03	BSA ≥1.7 m^2	0.20 (0.03 - 1.23)	0.08	BSA ≥1.57 m^2	0.21 (0.05 - 0.97)	0.04
User of Tylenol	3.93 (1.03 - 15.07)	0.04	User of Tylenol	3.38 (0.93 - 12.35)	0.07	User of Tylenol	2.84 (0.68 - 11.93)	0.15
Model III			Model III			Model III		
Women sex	0.92 (0.11 - 7.52)	0.94	Women sex	2.00 (0.34 - 11.84)	0.44			
Age	1.06 (0.98 - 1.15)	0.12	Age	1.06 (0.98 - 1.15)	0.12	Age	1.059 (0.97 - 1.15)	0.17
Weight	0.89 (0.81 - 0.99)	0.03	Weight ≥55	0.19 (0.04 - 0.83)	0.03	Weight ≥55	0.20 (0.05 - 0.87)	0.03
User of Tylenol	3.54 (0.95 - 13.21)	0.06	User of Tylenol	3.93 (1.02 - 15.2)	0.04	User of Tylenol	2.66 (0.64 - 11.12)	0.18

Anti-SARS-CoV-2 antibodies was measured using SG medical. BSA cut off was determined by median.
P-values are derived from adjusted logistic regression analysis.
SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; OR, odd ratio; CI, confidence interval; BMI, body mass index; BSA, body surface area.

of anti-SARS-CoV-2 antibodies levels between each diagnostic kit and at least three ELISA kits showed individual variations of anti-SARS-CoV-2-specific antibody levels.

SARS-CoV-2 is an enveloped, non-segmented positive-sense RNA virus with several structural proteins, including spike, envelop, membrane, and nucleocapsid. The spike protein contains an RBD that's responsible for recognizing the ACE2 cell surface receptor. The RBD of the SARS-CoV-2 spike protein strongly interacts with the human ACE2 receptor, leading to

endocytosis in the host cells of the lung and viral replication. Neutralizing antibodies mainly act against the RBD of the SARS-CoV-2 S protein, effectively blocking viral entry. In this study, three ELISA kits showed widely different individual variances in inhibition strength, whereas the inhibition range had a little individual variance in the Sugentech kit. Anti-SARS-CoV-2 antibodies levels were strongly correlated between 3 diagnostic kits (see **Figure 2**). These high correlations give strength to study results. Modest correlation was observed between the Sugentech kit and any other kit. This is perhaps related to the small dynamic range of neutralizing antibody titers as measured by this kit.

We investigated the correlation between anti-SARS-CoV-2 antibodies levels and associated factors using 4 different commercial ELISA kits. The SG Medical and HB Healthcare kits showed anti-SARS-CoV-2 antibodies levels were inversely correlated with weight, BMI, BSA, and weight/height. All four kits showed no association between adverse events and anti-SARS-CoV-2 antibodies level.

Further analysis showed that younger, lower body weight, BSA, and weight/height ratio have higher levels of anti-SARS-CoV-2 (SG medical kit). These features were similar in Genscript kit and HB healthcare kit. Lower BSA and use of Tylenol® had higher levels of anti-SARS-CoV-2 antibodies (Sugentech kit), and anti-SARS-CoV-2 antibody levels did not differ by systemic adverse event grade (all ELISA kits). At least three ELISA kits suggested differences in anti-SARS-CoV-2 antibodies according to various factors. These similar associations between factors and anti-SARS-CoV-2 antibodies levels provide convincing confirmation of our results.

We categorized anti-SARS-CoV-2 antibodies into binary upper and lower 50%. The upper antibody group showed lower values in BMI, body weight, BSA, and weight/height than the lower antibody group. In adjusted analyses, weight and BSA as continuous variables were negatively associated with anti-SARS-CoV-2 antibodies. Weight >55kg and BMI >22kg/m^2 had a negative association with upper 50% of anti-SARS-CoV-2 antibodies. Therefore, body weight and BMI can be used as surrogate markers to predict immune response of BNT162b2 mRNA vaccination (Pfizer, USA). Weight and BMI can be easily measured or calculated, so they can be used in predicting the efficacy of COVID-19 vaccination in clinical settings. Host intrinsic factors may contribute to immunoreactivity after vaccination, but their role in predicting vaccine efficacy has yet to be investigated. Even if host intrinsic factor is investigated in the future, blood sampling and testing intrinsic factors to predict immunoreactivity after vaccination has limitations in clinical application. In sub-analysis for women, weight >55kg (aOR: 0.20), BMI 22 kg/m^2 (aOR: 0.17), and BSA >1.57 m^2 (aOR: 0.21) had a negative association with upper 50% of anti-SARS-CoV-2 antibodies. This result is similar to those in the total population. In a large Japanese study (n = 2,435), antibody after COVID-19 vaccine was inversely associated with BMI in men, but not women [15]. However, the interval between blood sampling and the second vaccination was wide (15 – 103 days: median interval of 64 days). A variable sampling interval may produce biased results. In our study, sampling time of all participants is same (2 months after 2nd vaccination).

In a recent US cohort study, the vaccinated persons showed J-shaped associations between BMI and COVID-19 hospitalization and death after the second dose [16]. In a study using hospitalized patients with COVID-19, breakthrough cases showed that patients with lower antibody against SARS-CoV-2 were more likely to be solid organ transplant recipients, with higher need for ICU care, and higher mortality than patients with higher antibody titer [17]. The inverse association between BMI and antibody titer after vaccination, which

was suggested by our study and several previous studies [15, 18], may partially explain the negative association between BMI and clinical outcome in breakthrough COVID-19 infection (**Supplementary Fig. 3**).

Two-dose immunizations of mRNA vaccine yielded high levels of anti-SARS-CoV-2-specific antibody in 100% of participants in this study as in previous reports [11, 19]. Participants were young and middle-aged (22 – 54 years), so immunogenicity for mRNA vaccine may be relatively good. Persons younger than 39 years had higher anti-SARS-CoV-2-specific antibody than those older than 39 years. Women tended to have higher anti-SARS-CoV-2 antibodies than men. Differences in anti-SARS-CoV-2 antibodies by age and sex may be explained by weight differences. Younger age (<39 years) correlated with lower body weight and women had lower body weight than men. However, age and sex had no association with anti-SARS-CoV-2-specific antibody level in the adjusted analysis.

Systemic adverse events were reported as 80% and had no association with anti-SARS-CoV-2-specific antibody level after vaccination with BNT162b2 (Pfizer, USA). This result is compatible with a previous Korean report that adverse reactions to AZD1222 and BNT162b2 (Pfizer, USA) were not associated with anti-SARS-CoV-2 antibody [11]. Systemic adverse events may be associated with the immunologic properties of vaccines, physicochemical properties, and intrinsic host factors [20]. In this study, most systemic adverse events were grade 1–2, and the duration of systemic adverse events was ≤ 3 days in 98% of cases.

The primary strength of this study is the designation of factors associated with anti-SARS-CoV-2-specific antibody level after vaccination with BNT162b2 (Pfizer, USA). Body weight, BMI, and BSA can be used as surrogate markers for immunoreactivity after COVID-19 mRNA vaccination. Another strength is that the results were tested with four different ELISA kits. This study has several limitations. First, we did not evaluate baseline antibody levels. However, none of the participants had a history of COVID-19 infection and they constantly keep COVID-19 safety guideline in work place. Thus, participants had a very low risk of past infection of COVID-19. Second, only one vaccine was evaluated and other SARS-CoV-2 vaccines may yield different results. Third, participants were young and middle-aged persons, and it is unknown if the correlation of anti-SARS-CoV-2-specific antibody and weight or BMI is useful in elderly people. Fourth, we categorized BMI into two groups due to small sample size instead of underweight, normal, and overweight. Last, neutralization antibody titer could not explain all clinical protection (such as the protection from severe infection) [21] Thus, there is a limitation to explain the vaccine effectiveness in real-world.

In conclusion, anti-SARS-CoV-2-specific antibody level at 2 months after mRNA vaccination was inversely correlated with weight and BMI at least two different diagnostic kits in young and middle aged health population. Binary anti-SARS-CoV-2-specific antibody level was associated with categorized BMI, but not with continuous variable of BMI. However, binary anti-SARS-CoV-2-specific antibody level was associated with both categorized weight and continuous variable of weight. These results suggest that these factors, especially weight may be used as a surrogate marker to predict immune response of BNT162b2 mRNA vaccination (Pfizer, USA). Further investigation is needed to determine whether this correlation is sustained during a longer follow-up.

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SUPPLEMENTARY MATERIALS

Supplementary Table 1
Principal technical and analytical features of anti-SARS-CoV-2 antibodies immunoassays

Click here to view

Supplementary Table 2
Spearman's correlation between antibody detected by different diagnostic kits and demographic factors

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Supplementary Table 3
Level of anti-SARS-CoV-2 antibodies by categorical variables

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Supplementary Table 4
Difference of BMI and weight by age and sex

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Supplementary Table 5
Association between variables and binary value of anti-SARS-CoV-2 antibodies in women

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Supplementary Figure 1
Spline fit model for BMI, weight and BSA.

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Supplementary Figure 2
Adverse event after 2nd shot of BNT162b2 mRNA COVID-19 vaccine (n = 50).

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Supplementary Figure 3
Summary of this study and related study.

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