Original Article

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Antibody response of smokers to the COVID-19 vaccination: Evaluation based on cigarette dependence

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SUMMARY Smokers may have lower antibody titers after vaccination with a coronavirus disease 2019 (COVID-19) mRNA vaccine. However, to the best of our knowledge, no study has evaluated antibody titers after COVID-19 vaccination based on the level of smokers' cigarette dependence. In this study, we measured the level of serum anti-severe acute respiratory syndrome coronavirus 2 (anti-SARS-CoV-2) spike protein receptor-binding domain (S-RBD) immunoglobulin-G (IgG) by enzyme linked immunosorbent assay of 55 actively smoking Japanese social workers (firefighters, paramedics, and rescue workers) who had received two doses of the BNT162b2 vaccine. Further, we assessed their cigarette dependence using the Fagerstrom Test for Nicotine Dependence (FTND), measured their serum cotinine levels, and tested for their correlation with anti-RBD IgG levels. Serum anti-SARS-CoV-2 S-RBD protein IgG levels after BNT162b2 vaccination showed a significant negative correlation with FTND ($\rho = -0.426$, $p = 0.001$). In addition, serum cotinine level showed a significant positive correlation with FTND ($\rho = 0.470$, $p = 0.000$). However, no significant negative correlation was noted between serum cotinine and serum anti-SARS-CoV-2 S-RBD protein IgG levels ($\rho = -0.156$, $p = 0.256$). Our results suggest that smokers with strong cigarette dependence have inadequate anti-SARS-CoV-2 S-RBD protein IgG levels after COVID-19 mRNA vaccination.

Keywords SARS-CoV-2, COVID-19, mRNA vaccine, anti-RBD IgG level, cigarette dependence

1. Introduction

Since the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in 2019, the situation has evolved into an unprecedented global pandemic (*1*). In response to this challenging situation, the emergency use of vaccines BNT162b2/Pfizer and mRNA-1273/Moderna was approved by the US Food and Drug Administration in December 2020 as a prophylactic strategy against novel coronavirus disease 2019 (COVID-19) (*2,3*).

Both BNT162b2 and mRNA-1273 are mRNA vaccines are developed with the spike (S) protein as the target antigen. The S protein of SARS-CoV-2 is a major surface protein that binds with high affinity to angiotensin-converting enzyme 2 (ACE2 receptor) on human cells (*4*). Therefore, S proteins, especially the S1 receptor-binding domain (RBD), has been considered as a major target for neutralizing antibodies (*5*). Previous studies have highlighted the efficacy of these two mRNA vaccines in preventing COVID-19 and reducing the risk of severe disease (*6*).

In Japan, vaccination on priority with the COVID-19 mRNA vaccine began in early February 2021 for approximately 4.8 million healthcare workers, and in early April 2021, for approximately 36 million elderly people. Factors affecting low antibody titers after COVID-19 mRNA vaccination have been studied, including age (*7*), sex (male) (*8*), central obesity, and hypertension (*9*). One of the serious concerns reported is that smokers have lower antibody titers than non-smokers (*9,10*). Previous studies have shown that

smoking shortens life expectancy, increases overall health care costs, and contributes to reduced productivity (*11*). These studies clearly emphasize the detrimental effects of smoking on human health. Furthermore, suppressed immune function due to prolonged exposure to nicotine from smoking has also been reported (*12-14*). However, in addition to nicotine, cigarette smoke contains a very large number of toxic components, such as tar and carbon monoxide. Therefore, the mechanisms responsible for smoking-induced immune modulation after COVID-19 mRNA vaccination is necessary to be studied. Furthermore, the effect of cigarette dependence on antibody titers remains unclear.

Therefore, we measured the level of serum anti-SARS-CoV-2 spike protein receptor-binding domain (S-RBD) immunoglobulin-G (IgG) by enzyme linked immunosorbent assay (ELISA) of 55 actively smoking Japanese social workers (firefighters, paramedics, and rescue workers) who had received two doses of the BNT162b2 vaccine. It is important to evaluate the serum anti-SARS-CoV-2 response of first responders who may be in contact with people infected with COVID-19 or with suspected individuals during a pandemic.

The Fagerstrom Test for Nicotine Dependence (FTND) (*15*) is one of the most widely used scales for assessing cigarette dependence. It is a self-reported test and has been used in the scientific validation of nicotine dependence and its relation to genetic predisposition (*16*) in patients with cancer (*17*). Therefore, we adopted the FTND as a measure to assess dependence on cigarettes.

Many previous studies have explained that cigarette dependence is closely related to nicotine, one of the components of cigarettes (*18,19*). In addition, the effects of nicotine on immune mechanisms have been demonstrated in previous studies (*20,21*). However, nicotine has a short half-life, making it difficult to be used as a marker of exposure to cigarette smoke. On the other hand, cotinine, a metabolite of nicotine, has a long half-life of approximately 10-20 hours (*22,23*) and has been used in several studies to assess smoking status (*24,25*). Therefore, serum cotinine levels were used as an objective index in the present study.

The objective of this study was to assess the correlation between cigarette dependence and anti-RBD IgG levels after COVID-19 mRNA vaccination in smokers. To the best of our knowledge, this is the first study to evaluate the effect of smokers' cigarette dependence on their antibody response to the COVID-19 vaccination. We believe that this study will add new insights into the association between smoking and antibody titers after COVID-19 mRNA vaccination.

2. Materials and Methods

The participants of this study were explained in detail about the purpose of the study, methods involved, sample collection processes, and the management of personal information in the study in advance, and written informed consent was obtained. The study was performed in accordance with the principles of the Declaration of Helsinki. This study was approved by the Chubu University Ethics Review Board (Approval No.: 20200042).

2.1. Volunteer donor sample

We used a cross-sectional study design. The participants were social workers (firefighters, paramedics, and rescue workers) working at five fire stations located in a single city in Japan. We recruited participants by distributing a request form for research cooperation to the managers of each station. Subsequently, 55 active smokers were enrolled in the study. The participants had completed their second dose of BNT162b2 vaccine in mid-May 2021. All participants had a three-week interval between the first and second vaccination. We conducted blood collection in mid-June, *i.e.*, about four weeks after most participants received their second dose. This period of blood collection was between the fourth and fifth wave of COVID-19 epidemic in Japan. We collected blood from the fingertips of the participants using safety lancets, paying close attention to hygiene and infection control. The blood samples were centrifuged at 3,000 rpm for 5 min after standing for 30 min after collection, and the serum was frozen and stored at -20°C.

2.2. Survey

Participants were requested to fill a questionnaire with questions on age, gender, smoking classification, medical history, and drug history.

2.3. FTND

FTND was used to assess cigarette dependence. The FTND is a scale with a minimum score of 0 and a maximum score of 10, with scores assigned based on six factors. Our analysis showed that the FTND had a Cronbach's coefficient alpha of 0.636, showing a high reliability when compared to findings of previous studies (*26,27*).

2.4. Measurement of anti-SARS-CoV-2 S-RBD protein IgG levels

Serum anti-RBD IgG levels were measured using ELISA. The Anti-SARS-CoV-2 S-RBD protein Human IgG Kit (Proteintech Group, Rosemont, IL, USA) was used to measure serum anti-RBD IgG levels according to the manufacturer's instructions. This ELISA kit utilizes indirect ELISA as the measurement principle.

2.5. Cotinine concentration

Serum cotinine concentrations were measured using the Cotinine ELISA Kit (Abnova Corporation, Neihu District, Taipei, Taiwan) according to the manufacturer's instructions. The Cotinine ELISA Kit produces a calibration curve that relates cotinine concentrations by solid phase competition. The microplate reader used to measure absorbance was a POWERSCAN HT (Bio Tec Instruments, Winooski, VT, USA).

2.6. Statistical analysis

Descriptive statistical data are presented as the median (interquartile range: IQR) depending on the distribution. The Kruskal-Wallis test was used to evaluate statistical differences between groups for FTND, serum anti-RBD IgG and serum cotinine concentrations. Spearman's rank correlation coefficients were calculated to assess bivariate correlations. Two-sided *p*-values < 0.05 were considered statistically significant. IBM SPSS Statistics version 27 (IBM Corp., Armonk, NY, USA) was used for statistical analysis.

3. Results

3.1. Study population

A breakdown of age, sex and smoking equipment used is given in Table 1. All participants were male. None of the participants reported the use of e-cigarettes. With respect to medical history, two (3.6%) participants had history of COVID-19, two (3.6%) had hypertension, one (1.8%) had cardiac disease, and one (1.8%) had hyperlipidemia. As for oral medication use, six (10.9%) participants took antiallergic drugs, one (1.8%) took hyperlipidemia medication, and one (1.8%) participant took antihypertensive drugs. Neither medical history nor oral medication use was employed as an explanatory variable during statistical analysis, because none of these factors were found to affect serum anti-RBD IgG levels, serum cotinine, and FTND, and because there was a large difference among the samples compared (Data not shown).

Table 1. Volunteer donor profile

	n	%
Age group		
20-29	13	23.6
30-39	29	52.8
40-49	9	16.4
50-59	2	3.6
60-69	2	3.6
Sex		
Male	55	100
Female	0	0
Smoking device		
Cigarette	14	25.5
Heat-not-burn tobacco	29	52.7
Combination of cigarette and Heat-not-burn tobacco	12	21.8

3.2. Anti-RBD protein IgG levels

The median level of anti-RBD IgG was 15.5 μg/mL (11.3-36.5 μg/mL). Anti-RBD IgG levels neither showed any significant difference between the age groups (p = 0.286) (Figure 1A) nor between the smoking devices (p = 0.278) (Figure 2A). Therefore, age and smoking device were not used as explanatory variables affecting serum antibody titers in this study.

3.3. FTND and serum cotinine concentration

The median FTND was 3.0 (2.0-5.0). Comparison of FTND by age group showed no significant differences (p = 0.144) (Figure 1B). Comparison of FTND by smoking device showed no significant difference (p = 0.078) (Figure 2B).

The median serum cotinine concentration was 60.4 ng/mL (44.1-150.0). There was a large variance in serum cotinine levels in this population, and individual differences were observed. Serum cotinine concentration did not show significant difference between the age groups (p = 0.284) (Figure 1C) as well as smoking devices (p = 0.868) (Figure 2C). Therefore, age and smoking device were not used as explanatory variables affecting FTND and serum cotinine concentration in this study.

3.4. Correlations between FTND, anti-RBD IgG, and serum cotinine levels

FTND showed a significant negative correlation with anti-RBD IgG levels (ρ = -0.426, p = 0.001) (Figure 3A). By contrast, FTND showed a significant positive correlation with serum cotinine concentration (ρ = 0.470, p = 0.000) (Figure 3B). Being consistent with these findings, there was a weak negative correlation between serum cotinine concentration and anti-RBD IgG levels, but the correlation was not statistically significant (ρ = -0.156, p = 0.256) (Figure 3C).

4. Discussion

We examined smokers in their 20s to 60s who were vaccinated with BNT162b2 vaccine in Japan. First, we found that serum anti-RBD IgG levels after BNT162b2 vaccination was negatively correlated with FTND. Smoking is not only a factor that increases the risk of severity of COVID-19 (*28*), but is also clearly detrimental to antibody production after COVID-19 mRNA vaccination (*9,10*). Additionally, in the present study we observed a decrease in antibody production after COVID-19 mRNA vaccination among smokers, especially in case of strong cigarette dependence.

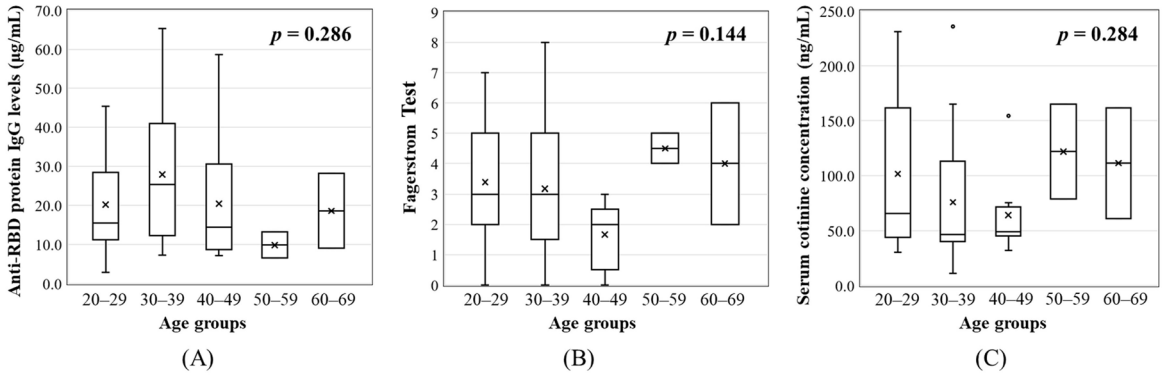

Figure 1. (A) Comparison of anti-severe acute respiratory syndrome coronavirus 2 (anti-SARS-CoV-2) spike protein receptor-binding domain (S-RBD) immunoglobulin-G (IgG) levels by age groups. (B) Comparison of the Fagerstrom Test by age groups. (C) Comparison of serum cotinine concentration by age groups. We compared anti-SARS-CoV-2 S-RBD protein IgG levels by age group showed no significant differences ($p = 0.286$) (Figure 1A). Comparison of the Fagerstrom Test by age group showed no significant differences ($p = 0.144$) (Figure 1B). Comparison of serum cotinine concentration by age group showed no significant difference ($p = 0.284$) (Figure 1C). All p values were derived from the Kruskal-Wallis test, $p < 0.05$ was considered statistically significant.

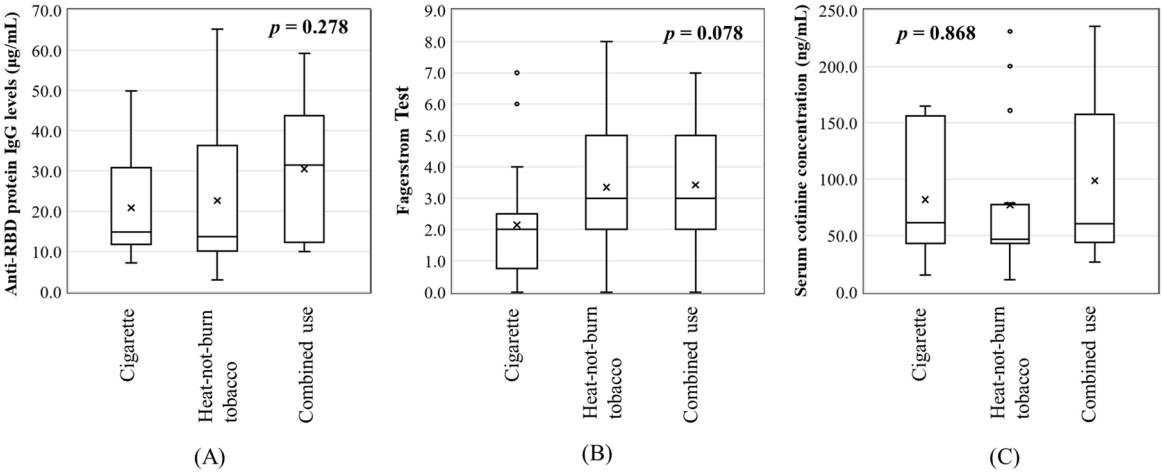

Figure 2. (A) Comparison of anti-severe acute respiratory syndrome coronavirus 2 (anti-SARS-CoV-2) spike protein receptor-binding domain (S-RBD) immunoglobulin-G (IgG) levels by smoking device. (B) Comparison of the Fagerstrom Test by smoking device. (C) Comparison of serum cotinine concentration by smoking device. We compared anti-SARS-CoV-2 S-RBD protein IgG levels by smoking device and found no significant difference ($p = 0.278$) (Figure 2A). Comparison of FTND by smoking device showed no significant difference ($p = 0.078$) (Figure 2B). Comparison of serum cotinine concentrations by smoking device category showed no significant difference ($p = 0.868$) (Figure 2C). All p values were derived from the Kruskal-Wallis test, $p < 0.05$ was considered statistically significant. FTND; Fagerstrom Test for Nicotine Dependence.

There was a significant positive correlation between FTND and serum cotinine levels, but there was no clear negative correlation between serum cotinine levels and serum anti-RBD IgG levels. These results indicate that the low antibody titers of the smokers after COVID-19 mRNA vaccination may be attributable not only to nicotine but also to other toxic substances which are contained in tobacco smoke.

To the best of our knowledge, no study has evaluated antibody titers after COVID-19 mRNA vaccination among smokers based on their level of cigarette dependence. We believe that this is the first study to demonstrate that there is a negative correlation between serum anti-RBD IgG levels and FTND after COVID-19 mRNA vaccination. This observation suggests that strong cigarette dependence may lead to reinforcement of smoking behavior, leading to a low antibody response after COVID-19 mRNA vaccination. However, the detailed mechanism is yet to be studied. COVID-19 mRNA vaccine has been reported to play an important role in reducing the risk and severity of the infection (*6*). Therefore, low antibody titers after COVID-19 mRNA vaccination in smokers with high cigarette dependence is an important concern during the pandemic. Another consideration is that smokers have unique characteristics that are influenced by their genetics, metabolism, physical and mental health status, as well as habits, personality, and lifestyle (*29*). Therefore, these characteristics may influence serum anti-RBD levels after COVID-19 mRNA vaccination. In addition, occupation of the participants and the type of mRNA vaccine administered were limited in

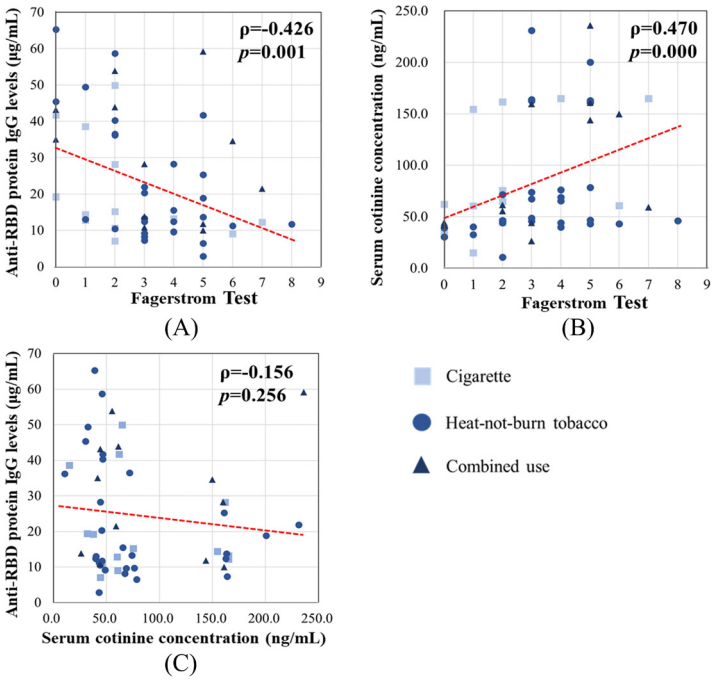

Figure 3. (A) Correlation between the Fagerstrom Test and anti-severe acute respiratory syndrome coronavirus 2 (anti-SARS-CoV-2) spike protein receptor-binding domain (S-RBD) immunoglobulin-G (IgG) level. (B) Correlation between Fagerstrom Test and serum cotinine concentration. (C) Correlation between serum cotinine concentration and anti-severe acute respiratory syndrome coronavirus 2 (anti-SARS-CoV-2) spike protein receptor-binding domain (S-RBD) immunoglobulin-G (IgG) level. FTND showed a significant negative correlation with anti-SARS-CoV-2 S-RBD protein IgG levels (ρ = -0.426, *p* = 0.001) (Figure 3A). Serum cotinine concentration showed a significant positive correlation with FTND (ρ = 0.470, *p* = 0.000) (Figure 3B). There was a negative correlation between serum cotinine concentration and anti-SARS-CoV-2 S-RBD protein IgG levels, but the correlation was not statistically significant (ρ = -0.156, *p* = 0.256) (Figure 3C). Values of ρ and *p* were derived from Spearman's test, $p<0.05$ was considered statistically significant. FTND: Fagerstrom Test for Nicotine Dependence.

this study. Furthermore, due to the nature of the study objectives, the establishment of a control group has challenging aspects. It is also necessary to take into account that the participants in this study were members of the fire department (firefighters, paramedics, and rescuers), who work hard around the clock and may have different circadian rhythms compared to other professionals. The results of the current study showed no significant differences in serum anti-RBD IgG levels and FTND between cigarette, heat-not-burn tobacco, and combined use groups. Therefore, we believe that concerns regarding low antibody titers after COVID-19 mRNA vaccination due to high cigarette dependence should be noted not only for cigarette smokers but also for heat-not-burn tobacco smokers and combination smokers. It is also possible that a history of COVID-19 could enhance antibody titers after vaccination (*30,31*). However, the two patients with a history of COVID-19 in this study were not treated as variables to be adjusted for, as there was no such trend.

Nicotine has effects on anxiety and stress reduction, pleasure, stimulation, and mood modulation (*32*), and may lead to cigarette dependence and reinforce smoking behavior. In the present study, there was a significant positive correlation between FTND and serum cotinine concentration, a metabolite of nicotine, which supports the results of previous studies, though the characteristics of the samples differed (*33*). We tested whether serum cotinine concentration also correlated negatively with serum anti-RBD IgG levels, as in the case of FTND. However, serum anti-RBD IgG levels after vaccination did not show a reliable negative correlation with serum cotinine levels.

The effects of nicotine on immune mechanisms have been shown in previous studies (*20,21*). In particular, it has been reported that prolonged exposure to nicotine through smoking can induce B cells that decrease antibody secretion, inhibit cell proliferation and development, and ultimately suppress normal immune function (*12,14*). However, cigarette smoke contains more than 4,500 components in its gaseous and particulate phases (*34*). Previous studies have shown that tobacco smoke affects a variety of host defense mechanisms (*20*), but due to the large number of toxic substances in tobacco, these mechanisms are not clearly understood (*35*). Our results suggest that when discussing the role of toxins in tobacco smoke in causing low antibody titers after COVID-19 mRNA vaccination, the risk factor may not be limited to nicotine. In addition, in our study, we did not find any significant difference in serum cotinine levels between groups according to the smoking device, suggesting that the type of smoking device may not have contributed to the present results. Japan has become an important market for companies

producing heat-not-burn tobacco (*36*), and 74.5% of the population we surveyed were either heat-not-burn tobacco smokers or combination smokers. However, none of the participants reported e-cigarette use; it was thus not included in the current study. Consistently, it is reported that nicotine concentrations in the smoke from cigarette paper and that from heat-not-burn tobacco (iQOS, distributed in Japan) were almost the same (*37*). In addition, there may be diurnal variation in blood cotinine concentration, and large individual differences were observed in this study. This may have been a factor that prevented a significant correlation between antibody titer and serum cotinine concentration.

In summary, our study represents the possibility that smokers who are heavily dependent on cigarettes may have particularly low antibody titers after COVID-19 mRNA vaccination. It also suggests that the factors influencing low antibody titers may not be limited to nicotine, but probably involve several other toxic substances. However, in the midst of the current COVID-19 pandemic, we do not necessarily argue that investigating those harmful substances is a top priority. This is because, although it can be assumed that there is some diurnal variation in blood cotinine levels, cigarette dependence itself is a persistent and constant factor for many individual smokers. Therefore, the key results we present highlight the possibility that the repeated smoking behavior, reinforced by a strong dependence on tobacco, may work against antibody titers after COVID-19 mRNA vaccination more adversely. This provides evidence that smokers with strong tobacco dependence may have insufficient protection against infection or severity of COVID-19 when vaccinated with COVID-19 mRNA vaccine.

There are several limitations to our study. First, this was a cross-sectional study and we were not able to identify any variation over time. Second, the assessment of SARS-CoV-2 antibody titers requires caution, as the assay used may yield multiple results (*38*). Third, because there are multiple measures to assess nicotine dependence other than the FTND used in this study, results may differ depending on the instrument used. Fourth, though previous studies have accounted for racial/ethnic differences in cotinine metabolism rates (*39,40*), the participants in our study were entirely of Japanese origin. Finally, though previous studies have shown that COVID-19 mRNA vaccines induce neutralizing antibody responses against three SARS-CoV-2 variants (*41*), the findings of the present study do not include the examination of neutralizing antibodies against SARS-CoV-2 variants.

5. Conclusions

In this study, we found that serum anti-RBD IgG levels were negatively correlated with FTND after BNT162b2 vaccination, while it showed no clear correlation with serum cotinine levels. These results suggest that repeated smoking behavior due to strong cigarette dependence may lead to low antibody titers after COVID-19 mRNA vaccination, and that the factors affecting low antibody titers after COVID-19 mRNA vaccination in cigarettes may not be limited to nicotine.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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