

The relationship between immunological age determined by Immune Age Index (IMMAX) and response to vaccination against SARS-CoV-2

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Abstract

Immunosenescence contributes to reduced vaccine efficacy in aging populations, but chronological age is an imperfect proxy for the underlying immunological decline. We investigated whether the Immune Age Index (IMMAX), a validated surrogate for the IMM-AGE metric, better predicts vaccination outcomes than chronological age. Using the unique setting of primary SARS-CoV-2 vaccination in immunologically naive individuals, we analyzed anti-spike RBD antibody titers and IMMAX in 658 fully vaccinated adults, including a subgroup of 91 professional firefighters. Both chronological age and IMMAX negatively correlated with antibody titers. Notably, women exhibited IMMAX values matching those of men roughly eight years younger and correspondingly showed higher antibody responses across the entire age range, indicating a sex-based immunological advantage independent of age. In the subgroup of 353 participants vaccinated twice with BNT162b2, our analysis revealed that while antibody titers declined linearly with chronological age, they remained stable across a range of lower IMMAX values before declining beyond an equivalent-years-of-life value of about 60. Professional firefighters displayed significantly lower IMMAX scores than age-, sex-, and lifestyle-matched controls, corresponding to an immune age advantage of 8.24 years. More importantly, they also showed significantly higher antibody titers compared to matched controls. These findings establish IMMAX as a robust, readily measurable biomarker that captures individual variation in immune competence beyond chronological age.

Introduction

With aging, but also due to continuous stimulation in chronic infection, the functionality of the immune system declines. This process is called immunosenescence and is characterized by an increasing low-grade inflammation, paralleled by distinct shifts in the composition of immune cell subsets such as naive vs. memory T cells and altered expression levels of certain surface markers, that are involved in the modulation of immune cell function. For example, the co-stimulating receptor CD28 is lost during immune cell aging, while expression of inhibitory receptors like KLRG1 or PD-1 is increased (reviewed in ^{1,2}). These changes mean that the immune system of older people responds less effectively to challenges such as infections, which can more frequently lead to a severe course of disease ³. In this context, a reduced or even impaired response to various vaccinations has also been described in elderly people. For this reason, a number of vaccines have now been developed that are designed to stimulate the aging immune system more strongly and to induce an effective immune response, especially in older or immunocompromised individuals. These new vaccines either contain a higher concentration of influenza antigens ⁴⁻⁶, protein-conjugated bacterial antigens in case of pneumonia ^{7,8} or adjuvants in case of Herpes zoster ⁹ or influenza ⁴, to boost the immune response.

Many studies have investigated how chronological age can influence the vaccination success and they have shown a correlation between age and vaccine-induced antibody titers ¹⁰⁻¹². However, chronological age is only a proxy for immunological age and individuals with the same chronological age can show vastly different degrees of immunosenescence ¹³. Therefore, immunological age may be a better

predictor of vaccination efficacy than chronological age. The construct 'immune age' tries to reflect age-related changes in the immune system, which vary between individuals and do not necessarily parallel chronological age. There are currently different approaches to investigate the relationship between chronological age and immune age¹⁴. The application of 'omics' approaches to determine biological age in different organs and tissues is reviewed in Kočar et al.¹⁵. However, many of these approaches are very resource-intensive. Alpert et al. used a complex approach to determine the IMM-AGE score in a longitudinal analysis of more than 30,000 markers using multiple 'omics' technologies including flow cytometry¹⁶. Based on this IMM-AGE score, we developed our IMMAX metric¹⁷. This approach uses a fast and simple flow cytometry-based minimal panel to provide information on the correlation between chronological and immunological age that are comparable to the more complex IMM-AGE score. IMMAX, as a measure of biological age, was a better predictor for cardiorespiratory fitness than chronological age¹⁷, predicted low work ability¹⁸, and was shown to be a robust biomarker that is independent from the influence of the circadian rhythm of immune cell concentrations in peripheral blood¹⁹. The integration of more than 1,600 individual data has also enabled us to convert the IMMAX score into equivalent years of life (EYOL)¹³.

The first vaccinations against SARS-CoV-2 resulted in the rare situation that individuals were vaccinated against a pathogen to which they were immunologically naive, meaning that the initial situation was identical for all individuals tested. Thus, we took advantage of the unique situation during the early corona pandemic to study the response to some of the vaccines available at that time²⁰ dependent on age and immune age in individuals immunologically naive to SARS-CoV-2.

Our results show a strong correlation of antibody titers with immunological and chronological age. Interestingly, the IMMAX scores of men matched those of women who were about eight years older, making females immunologically younger. Antibody titers were also higher in women, suggesting an additional beneficial influence of sex on immune function. Interestingly, the IMMAX score of a cohort of 91 professional firefighters was lower than that of matched controls and the immunological age of male firefighters was comparable to that of women of the same age. More importantly, these immunologically younger professional firefighters also had higher antibody titers, suggesting that immunological age as determined by IMMAX can be used as a biomarker or the outcome of vaccination responses.

Materials and Methods

Participants

Blood collection took place between July and November 2021. We collected blood samples from 724 adult participants who were fully vaccinated against SARS-CoV-2 for at least 4 weeks. Samples were taken from venous blood or capillary blood from the fingertip into appropriate tubes (Monovettes or Microvettes with EDTA, Sarstedt). Plasma was stored at - 80°C until use. All participants gave written informed consent. Further, participants answered questionnaires regarding demographic, anamnestic

and anthropometric data and gave information about applied SARS-CoV-2 vaccination strategy and previous infection with SARS-CoV-2. The study was approved by the ethics committee of the Leibniz Research Centre for Working Environment and Human Factors (approval #198).

Flow Cytometry and Determination of IMMAX

All antibodies and reagents were individually titrated to determine the optimal dilution. All reagents, antibodies and dilutions are listed in suppl. table 1. For each sample, 100 µl fresh whole blood were stained with the live/dead stain and the antibody cocktail for 20 min at RT in the dark. Then, red blood cells were lysed for 10 min with 1 mL of FACS Lysing Solution (Waters Biosciences). Samples were centrifuged, supernatant was discarded, and cells were resuspended in 250 µL FACS buffer (PBS + 2% FCS). Samples were acquired on a BD LSRFortessa™ Cell Analyzer. Data were analyzed using FlowJo™ v10.10 (BD Life Sciences). Gating strategy was published recently ¹³. We determined the following relative cell frequencies: NK/T cell ratio, CD4+/CD8 + T cell ratio, memory/naïve CD4 + and CD8 + T cell ratios, CD28- CD8 + T cells. IMMAX values were calculated from the relative cell frequencies as described and converted into equivalent years of life (EYOL) ¹³.

Determination of anti-spike RBD antibodies in plasma

Concentration of anti-spike RBD antibodies in plasma was determined essentially as described ²¹. Briefly, the RBD sequence (spike glycoprotein amino acids 319–541) of the Wuhan strain of SARS-CoV-2 with a C-terminal HIS-tag was expressed in HEK 293-F cells and purified on a HisTrap Excel column using an ÄKTAexpress purification system. Ninety-six well flat bottom plates (maxisorp; Nunc) were coated with 3 µg/mL of SARS-CoV-2 spike RBD overnight at 4°C. Plates were washed, blocked with Biolegend ELISA diluent, and then incubated with plasma samples diluted in blocking buffer. The S Antibody (humanized anti-spike antibody by Dianova/Cusabio, Stock concentration: 0.3 mg/mL) was used as positive control (1:5,000 final dilution) and calibrator (1:40,000 final dilution). As a secondary antibody, HRP conjugated anti-human IgG (Dianova), was used and signals were detected with 1 Step Ultra TMB (Pierce). The relative absorbance was calculated using the formula: (sample-negative control)/(calibrator-negative control). Values > 1 are positive, values < 1 but > 0.5 are borderline and should be repeated, and values < 0.5 are negative at a 1:100 dilution of the sample. To calculate antibody titers in BAU/mL, the samples were diluted from 1:100 to 1:12,500 and the dilution was calculated for which the relative absorbance would be 1. To do so, hyperbolic curves with the formula $Y = B_{max} * X / (K_d + X)$ were fitted using Graph Pad Prism (version 9). Simultaneously, the WHO Standard 20/136, which is defined to have 1,000 BAU/mL was also measured to calculate a correction factor to express the dilution values as BAU/mL.

Determination of CMV seropositivity in plasma

Qualitative determination of IgG-class antibodies against Cytomegalovirus (CMV) in plasma was performed using the Cytomegalovirus (CMV) IgG ELISA (IBL International) according to the manufacturer's instructions.

Data analysis and statistics

Bivariate associations with anti-RBD titers were assessed by pairwise two-sided Wilcoxon tests with p-values adjusted for multiple testing by the Bonferroni procedure for categorical predictors, and by Pearson's correlation coefficient for continuous predictors, respectively. Multivariate associations of log titer with continuous and categorical predictors were assessed by multiple linear regression and by cubic regression splines relaxing the linearity assumption²². Effect estimation and statistical comparison of firefighters to the general population were performed by g-computation after propensity score matching²³. Computations were carried out using R version 4.6²⁴.

Results

Participants and study design

We collected blood samples from 724 participants who were fully vaccinated against SARS-CoV-2 for at least 4 weeks. Participants received one (Ad26.COV2.S) or two doses of the vaccines available at the time of enrollment (Fig. 1). We excluded 66 participants as they did not meet all inclusion criteria, and 658 participants were included in the analysis. For more detailed analyses, samples were further stratified into general population (n = 567) vs. professional firefighters (n = 91). Moreover, we performed a subgroup analysis of samples from 353 participants of the general population vaccinated twice with the BNT162b2 vaccine. Demographic and epidemiological characteristics of the included 658 participants are summarized in Table 1.

Table 1

Demographic characteristics, anti-RBD titers and predictors of participants eligible for analysis from the general and firefighter populations, and from the subgroup vaccinated twice with the BioNTech/Pfizer vaccine BNT162b2 (BB).

Characteristic	General Population	BB Subgroup	Firefighters
	N = 567 ¹	N = 353 ¹	N = 91 ¹
Age (years)	53 (32, 63)	53 (32, 62)	39 (30, 53)
Sex			
<i>males</i>	235 (41%)	136 (39%)	84 (92%)
<i>females</i>	332 (59%)	217 (61%)	7 (7.7%)
Body height (cm)	172 (165, 180)	171 (165, 178)	182 (176, 187)
Body mass (kg)	76 (64, 89)	74 (63, 87)	84 (78, 95)
BMI category			
<i>normal</i>	274 (48%)	177 (50%)	36 (40%)
<i>overweight</i>	193 (34%)	116 (33%)	47 (52%)
<i>obese</i>	100 (18%)	60 (17%)	8 (8.8%)
CMV seropositive	235 (41%)	140 (40%)	26 (29%)
Smoker	176 (31%)	103 (29%)	35 (38%)
With chronic disease	203 (36%)	127 (36%)	15 (16%)
Vaccine			
<i>BB</i>	353 (62%)	353 (100%)	35 (38%)
<i>AA</i>	61 (11%)	0 (0%)	10 (11%)
<i>AB</i>	68 (12%)	0 (0%)	38 (42%)
<i>AM</i>	13 (2.3%)	0 (0%)	0 (0%)
<i>MM</i>	59 (10%)	0 (0%)	8 (8.8%)
<i>JJ</i>	13 (2.3%)	0 (0%)	0 (0%)
Months vaccinated	4.07 (2.90, 4.97)	4.40 (3.03, 5.17)	5.07 (4.40, 5.37)
Notes: BB: BNT162b2 (BioNTech/Pfizer) + BNT162b2 (BioNTech/Pfizer); AA: AZD122 (AstraZeneca) + AZD122 (AstraZeneca); AB: AZD122 (AstraZeneca) + BNT162b2 (BioNTech/Pfizer); AM: AZD122 (AstraZeneca) + mRNA-1273 (Moderna); MM: mRNA-1273 (Moderna) + mRNA-1273 (Moderna); JJ: Ad26.COV2.S (Johnson & Johnson). BMI: body mass index, CMV: cytomegalovirus, EYOL: IMMAX expressed as equivalent years-of-life scale, IMMAX: approximation to IMM-AGE metric by principal component regression.			

Characteristic	General Population	BB Subgroup	Firefighters
	N = 567 ¹	N = 353 ¹	N = 91 ¹
anti-RBD (BAU/ml)	586 (207, 1,526)	635 (238, 1,438)	576 (262, 1,264)
Immune age markers			
IMMAX	0.46 (0.38, 0.56)	0.45 (0.37, 0.56)	0.40 (0.34, 0.48)
EYOL (years)	49 (33, 69)	47 (30, 69)	36 (24, 52)
¹ Median (Q1, Q3); n (%)			
Notes: BB: BNT162b2 (BioNTech/Pfizer) + BNT162b2 (BioNTech/Pfizer); AA: AZD122 (AstraZeneca) + AZD122 (AstraZeneca); AB: AZD122 (AstraZeneca) + BNT162b2 (BioNTech/Pfizer); AM: AZD122 (AstraZeneca) + mRNA-1273 (Moderna); MM: mRNA-1273 (Moderna) + mRNA-1273 (Moderna); JJ: Ad26.COV2.S (Johnson & Johnson). BMI: body mass index, CMV: cytomegalovirus, EYOL: IMMAX expressed as equivalent years-of-life scale, IMMAX: approximation to IMM-AGE metric by principal component regression.			

IMMAX Analysis of the study population

We quantified the response to vaccination against SARS-CoV-2 by measuring the anti-spike RBD antibody titers in plasma samples. As expected, chronological age was negatively correlated with anti-spike RBD antibody titers (Fig. 2A). To test if immunological age also influences the vaccination outcome, we determined the IMMune Age index IMMAX by flow cytometry as described ¹³. We validated the IMMAX score by comparison of the IMM-AGE metric from Alpert et al. and our IMMAX score for the original data set ¹⁶ with the approximations in this study (IMMAX.VAC). All metrics showed comparable correlation with chronological age on the different data sets and thus confirmed the applicability of our IMMAX score for the vaccination data set (Fig. 2B). The IMMAX of women matched those of men who were about eight years younger (Fig. 2C), confirming previous findings that women are immunologically younger than males of the same chronological age ¹³. Chronic CMV infection is known to be involved in the induction of immune senescence ²⁵. We therefore analyzed the effect of CMV seropositivity on the correlation of chronological age and IMMAX for males and females (Fig. 2D). CMV seropositivity in both sexes strongly correlated with an aged immune phenotype, as reflected by increased IMMAX values. After this successful validation, we analyzed the effect of immunological age on anti-spike RBD antibody titers. We found a clear negative correlation of IMMAX with anti-spike RBD antibody titers in our cohort (Fig. 2E).

Bivariate analysis of vaccination strategy and epidemiological factors

The different vaccines and vaccination protocols induced varying amounts of antibodies with participants vaccinated twice with mRNA-1273 showing highest titers while participants receiving a

single dose of Ad26.COV2.S had lowest titers of anti-spike RBD antibodies (Fig. 3A). Further, the antibody titers decreased with increasing time since vaccination in all participants independent of vaccination strategy (Fig. 3B and supplementary Fig. 1). Using bivariate analysis, we found significant influence of additional demographic and epidemiological parameters on anti-spike RBD titers. Female participants showed higher response to vaccination (Fig. 3C), while obese BMI (Fig. 3D), smoking (Fig. 3E), or chronic disease (Fig. 3G) were associated with lower antibody titers. Interestingly, CMV seropositivity did not significantly affect vaccination response in the general population (Fig. 3F).

Multivariate analysis of epidemiological factors

Multiple regression analysis identified several epidemiological factors to predict anti-spike RBD antibody titers (Fig. 4A). In addition to expected factors such as chronological age, smoking, or vaccination strategy, female sex was positively associated with higher antibody titers. Obesity, but not overweight BMI, was found to have a negative effect on vaccination response. When we analyzed the influence of body weight in relation to sex (Fig. 4B), we found no sex differences in normal weight participants. Overweight BMI or obesity also did not affect antibody titers in females. However, male participants showed decreased titers compared to females in these conditions, and anti-spike RBD antibody titers of obese males were significantly decreased compared to normal weight males. Anti-spike RBD titers correlated with chronological (Fig. 4C) and immunological (Fig. 4D) age for both sexes. Notably, female participants showed higher antibody titers across the entire age range, both in terms of chronological age and IMMAX.

Differential effects of chronological and immunological age on vaccination outcome

While antibody titers induced by different vaccination strategies were highly variable (Fig. 2A), also the age distribution of participants receiving different vaccines was significantly different (supplementary Fig. 2). Twice AZD122 vaccination strategy was mainly applied to elderly subjects with mean age (SD) of 63 (9) years, while the mean age of participants vaccinated twice with mRNA-1273 or once with Ad26.COV2.S was 39 (14) and 38 (13) years, respectively. These differences in chronological age were paralleled by IMMAX. In contrast, BNT162b2 was given twice to a majority of subjects of all age groups. Therefore, we performed a subgroup analysis and focused on samples from participants vaccinated twice with BNT162b2 ($n = 353$). We re-analyzed the interaction of chronological and immunological age with antibody titers by regression spline functions fitted to the log-transformed anti-RBD titers for the BNT162b2 vaccinated subgroup as generalized additive model (GAM) relaxing the linearity assumption. As before, we found a linear decrease of anti-spike RBD antibody titers with increasing chronological age (Fig. 5A). However, when looking at immunological age, the antibody titers remained at a plateau until the IMMAX value exceeded a certain level, and from then on decreased in a linear trend (Fig. 5B). We recently established an IMMAX derivative equivalent years of life (EYOL), which expresses the

immunological age in an equivalent value of age¹³. Notably, the EYOL value at the turning point is about 60 years, an age that is described as the beginning of weakening of the immune system²⁶.

Professional firefighters have a lower immune age

Our cohort also included 91 professional firefighters, whose test results differed to some extent from those of the general population. When we compared data from firefighters to samples matched for chronological age, sex, vaccine type, time since vaccination, smoking status, and health status from the general population, the immune age of firefighters was about 8.24 years younger and thus matched the immune age difference between men and women in the general population (Fig. 6A). More importantly, when comparing the anti-spike RBD antibody titers between the firefighters and matched controls from the general study population we found that the immunologically younger firefighters also had significantly higher antibody titers (Fig. 6B).

Discussion

With the first vaccinations against the novel SARS-CoV-2, we had the unique opportunity to study the effects of these vaccines on immunologically naive individuals. This allowed us to generate a particularly clean data set that did not contain any noise from previous infections or vaccinations. We included subjects who were considered fully vaccinated with one of the vaccination strategies available at that time. We determined vaccination success based on the concentration of anti-spike RBD antibodies in an assay we developed²¹. The participants showed very different levels of response to the different vaccines. This has already been shown in earlier studies and has led to adjustments in vaccination strategies²⁰.

We found correlations of both chronological age and IMMAX with antibody titers, demonstrating that immunological age is an important factor for the success of immunization strategies. A previous study used a modified version of our IMMAX score to demonstrate that PBMCs from individuals with a higher IMMAX score produced fewer antibodies when exposed to recombinant SARS-CoV-2 Spike protein in vitro²⁷, however, they did not investigate in vivo vaccination responses. In our cohort, female participants across the entire age and IMMAX range showed a higher vaccine response. This is consistent with previous studies showing more efficient immune response in female subjects²⁸. In addition to genetic factors and sex hormones²⁹, also behavioral differences³⁰ probably play a role for the different vaccination outcome. A potentially healthier lifestyle among women is also being discussed. It is interesting to note that we found a significant negative influence of obesity on antibody titers only in men, which suggests that differences in body composition of males and females³¹ may also have a different impact on the immune function. However, since we have only determined the BMI here, a possible effect of body composition needs to be examined in more detail. As expected, smoking had a negative influence, meaning that IMMAX can integrate the influence of various epidemiological

factors on immune age. This will help to understand which factors are responsible for the vaccination success and how the interaction of a multitude of factors affects immune function.

Due to the varying responses, we focused some of our analyses on subjects who had been vaccinated twice with the BNT162b2 vaccine. While we found a linear relationship between vaccine response and chronological age, we could also show that the anti-RBD titer did not decrease linearly in relation to immune age but remained at a plateau over a wide range of the IMMAX score and started to decline at an EYOL value of about 60 years. This is particularly noteworthy as many recommendations for the use of modified vaccines for the elderly set the age of 60–65 years as the lower limit ²⁶. The fact that our IMMAX score was able to define a tipping point that corresponds to those long-term empirical values makes IMMAX a potential biomarker that can be easily used to determine the efficacy of vaccines in different immune age groups.

We did find a higher immune age in CMV seropositive subjects across the entire IMMAX range. This indicates that, in line with previous studies (reviewed in ³²), we were also able to demonstrate an influence of chronic CMV infection on immune aging in our cohort. However, the specific effect of CMV does not appear to have any influence on the vaccine response, which is in line with a recent study showing that CMV seropositivity does not interfere with CD8 T cell responses to SARS-CoV-2 infection ³³. Interestingly, in a recently published study, we did not observe an increase in IMMAX values in *Toxoplasma gondii*-positive subjects, suggesting that chronic infection with this eukaryotic parasite may cause other changes in the immune system that are not captured by the parameters defining the IMMAX score ³⁴.

Our cohort also included 91 professional firefighters, whose values for IMMAX were lower than the values of matched controls. The effect was of similar magnitude as the difference between women and men of the same age in the general population. In a previous study, we could show that cardiorespiratory fitness is a good predictor for IMMAX and that better fitness is associated with a lower immune age ¹⁷. Several studies demonstrated that improving physical fitness through regular exercise can result in alterations in the immune system ³⁵. Notably, many of those changes were observed in various T cell subpopulations ³⁶, which are also included in our IMMAX score. It is therefore possible that the better physical fitness of professional firefighters could play a role in the improved IMMAX values. More importantly, these lower IMMAX values were also correlated with higher vaccine-induced antibody titers compared to controls that were matched for chronological age, sex, vaccine type, time since vaccination, smoking status, and health status, arguing that immunological age is an important predictor of vaccine responses, independently of chronological age or other life-style factors. This is further supported by the finding that maintaining physical fitness during aging, which may affect the immunological age, also improves the response to vaccination against SARS-CoV-2 in older people ³⁷.

Our study also has several limitations. As the design was cross-sectional, we cannot infer causal relationships between fitness, lifestyle and immune age or vaccine-induced antibody titers. We cannot exclude a possible healthy worker selection bias in our firefighter cohort. As many data (BMI, smoking,

chronic disease etc.) were self-reported, we cannot exclude potential misclassifications. Finally, our assessment of the COVID-19 vaccination success is limited to anti-RBD binding antibodies, and we did not assess virus neutralization or T cell response data.

The correlation between immune age and vaccine responses against SARS-CoV-2 makes the determination of the immunological age an important tool to predict immune responses. The IMMAX is an easily accessible measure for immune aging and can be determined by a rather simple flow cytometric analysis of fresh blood or frozen PBMCs, allowing for the easy incorporation of IMMAX as a potential biomarker for immune age in future studies.

Declarations

Competing Interests

CW has received lecture fees from or participated in advisory boards for BioNTech, Biotest, GSK, Moderna, Novavax, Pfizer, and Sanofi but declares no non-financial competing interests. All other authors declare no financial or non-financial competing interests.

Author Contribution

MC and CW conceptualized the study. MC and DU designed and performed the experiments. NW analyzed the blood samples. MC and PB analyzed and visualized data. CW administrated and supervised the study. MC, PB and CW wrote the manuscript. All authors read and approved the final manuscript.

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Data Availability

The data and analysis scripts for reproducing the results presented in this study are available from https://osf.io/yskza/overview?view_only=f5aa5fcbfbf5478fb894b518d2036a6c for peer review. The link will be replaced by a permanent public identifier for publication.

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Figures

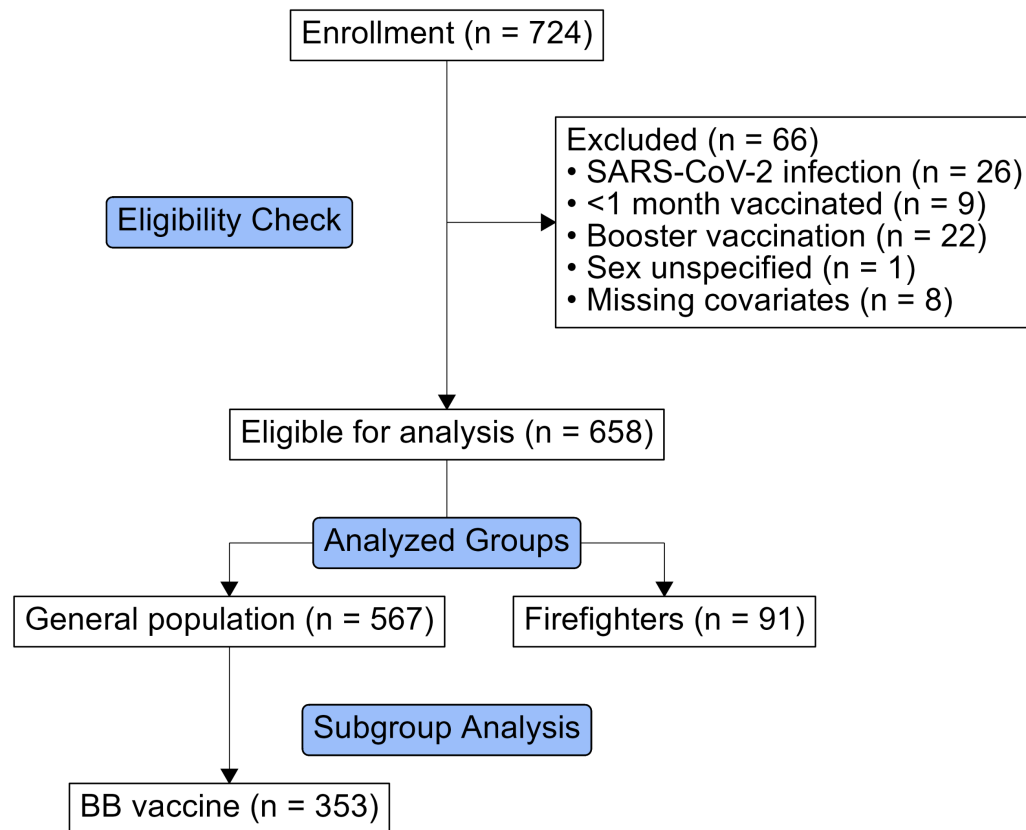


Figure 1

Study enrollment of participants, check for eligibility and definition of study group from the general and firefighter populations for analysis, and of the subgroup vaccinated twice with the BioNTech/Pfizer vaccine BNT162b2 (BB).

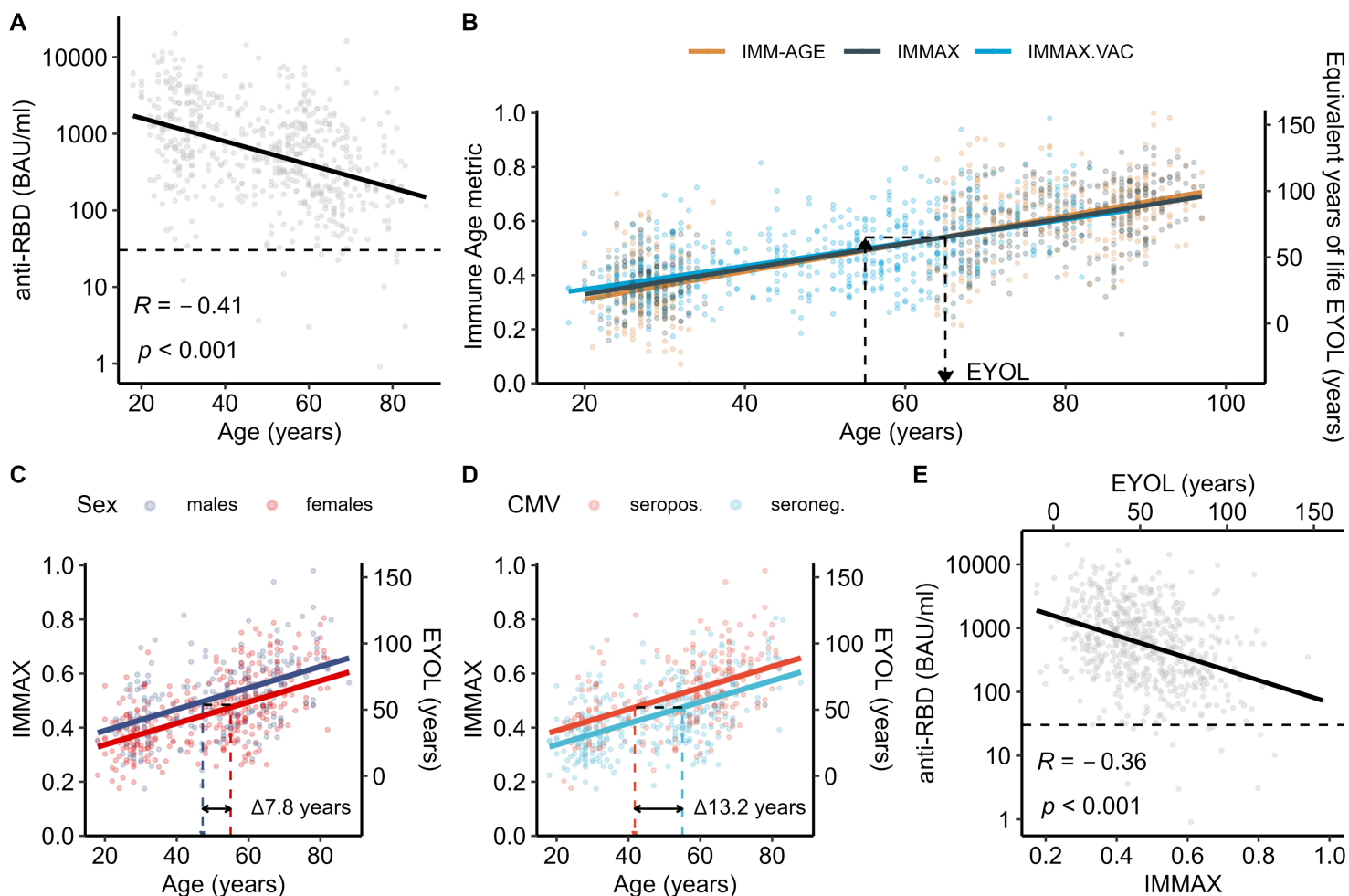


Figure 2

(A) Bivariate association of anti-RBD titers of the eligible observations from the general population (N=567) with chronological age. The dashed horizontal line refers to the lowest effective titer of 30.3 BAU/ml. Statistical significance assessed by Pearson's correlation coefficient (R). (B) IMM-AGE metric and its approximation IMMAX for the original data¹⁶ related to chronological age compared to the approximations in this study (IMMAX.VAC) with arrows visualizing the usage of the IMMAX regression line for re-scaling the metric as equivalent years-of-life (EYOL) shown on the secondary axes. (C) IMMAX depending on chronological age for subgroups defined by sex, and (D) by CMV status with regression lines and horizontal shift (Δ) determined by ANCOVA. (E) Correlation of anti-RBD titers with immune age assessed by the IMMAX metric.

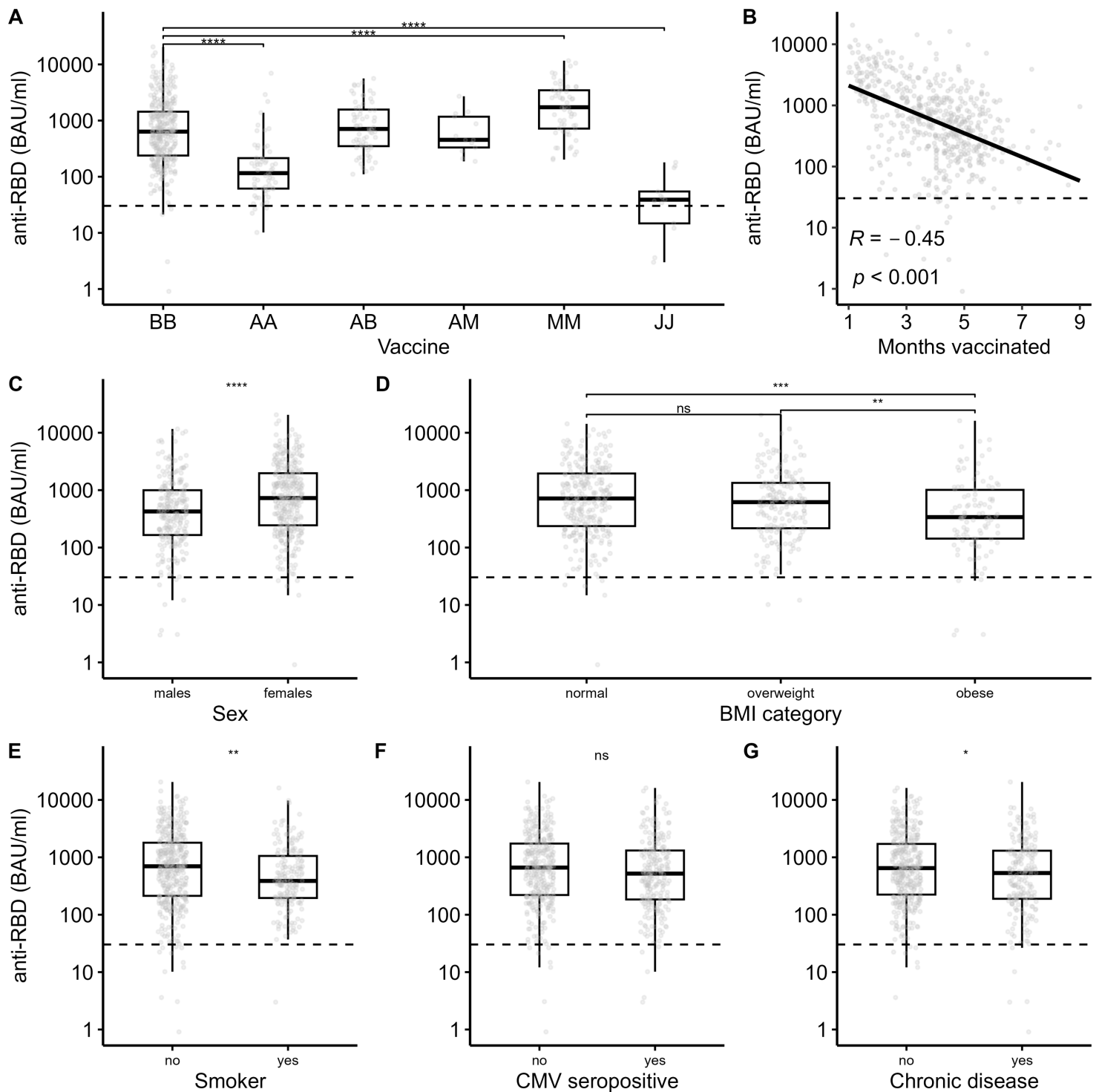


Figure 3

Bivariate associations of anti-RBD titers of the eligible observations from the general population (N=567) with (A) the vaccine combinations, (B) time since vaccination, (C) sex, (D) BMI, (E) smoking habits, (F) CMV status, (G) health status. Statistical significance (****: $p < 0.0001$, ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, ns: $p \geq 0.05$) assessed by pairwise Wilcoxon tests with p-values adjusted for multiple testing for categorical predictors, and by Pearson's correlation coefficient for continuous predictors, respectively. BB: BNT162b2 (BioNTech/Pfizer) + BNT162b2 (BioNTech/Pfizer); AA: AZD122 (AstraZeneca) + AZD122

(AstraZeneca); AB: AZD122 (AstraZeneca) + BNT162b2 (BioNTech/Pfizer); AM: AZD122 (AstraZeneca) + mRNA-1273 (Moderna); MM: mRNA-1273 (Moderna) + mRNA-1273 (Moderna); JJ: Ad26.COVS.2.S (Johnson & Johnson).

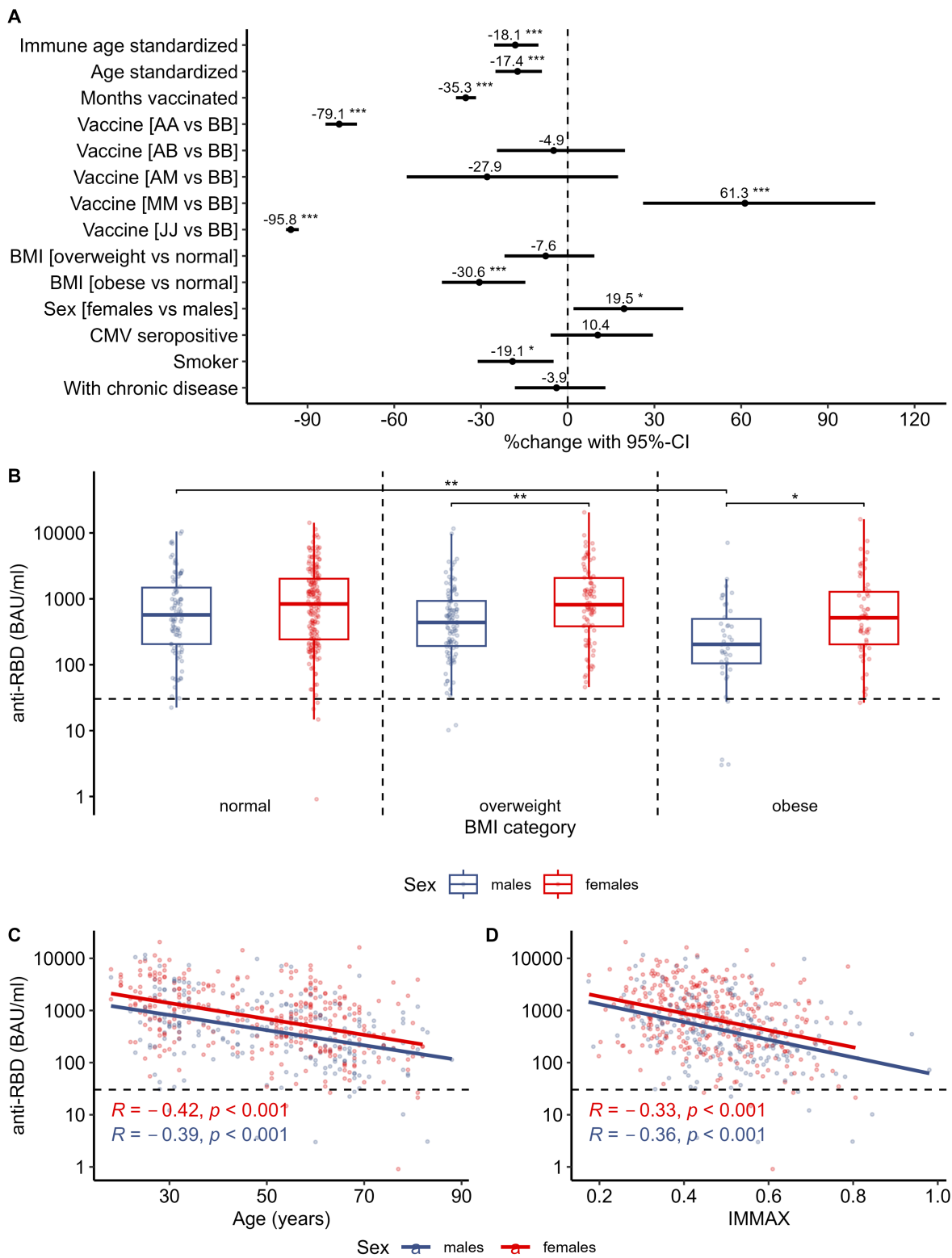


Figure 4

(A) Estimates from multiple regression analysis predicting log titer by continuous and categorical predictors expressed as %change calculated from the regression coefficients β as $\%change=100*(\exp(\beta)-1)$ (B) Interaction of sex with obesity, chronological age (C), and the immune age metric IMMAX (D) concerning anti-RBD titers of N=567 participants from the general population indicated by Pearson correlation coefficients (R, panels C, D) and pairwise Wilcoxon test results (panel B) with p-values adjusted for multiple testing. BB: BNT162b2 (BioNTech/Pfizer) + BNT162b2 (BioNTech/Pfizer); AA: AZD122 (AstraZeneca) + AZD122 (AstraZeneca); AB: AZD122 (AstraZeneca) + BNT162b2 (BioNTech/Pfizer); AM: AZD122 (AstraZeneca) + mRNA-1273 (Moderna); MM: mRNA-1273 (Moderna) + mRNA-1273 (Moderna); JJ: Ad26.COV2.S (Johnson & Johnson).

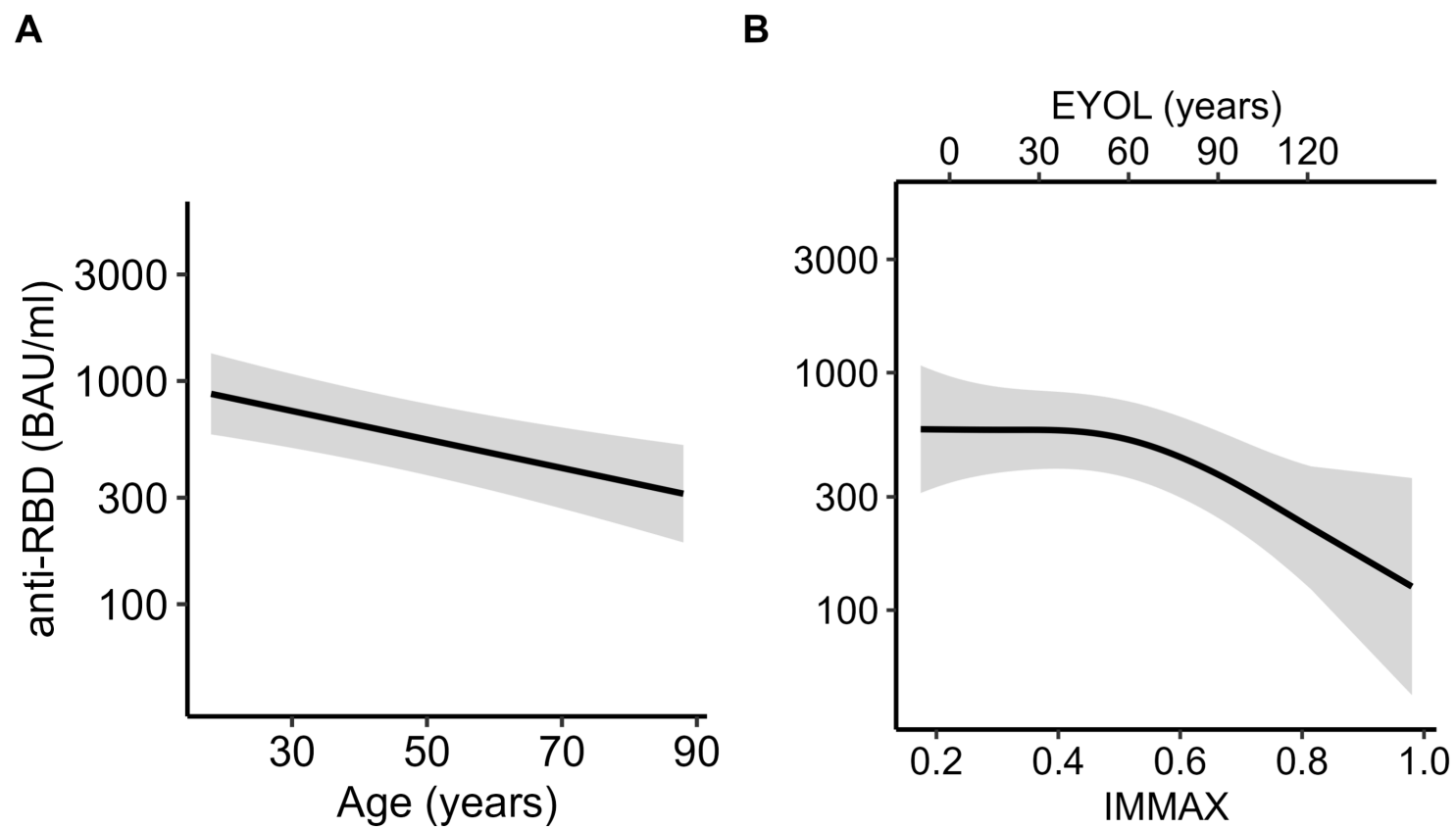


Figure 5

Relation of anti-RBD titers with (A) chronological age, and (B) immune age metric IMMAX, with values re-scaled as equivalent years-of-life (EYOL) shown on the secondary x-axis, assessed by regression spline functions fitted to the log-transformed anti-RBD titers for the subgroup data from N=353 participants vaccinated twice with BioNTech/Pfizer BNT162b2 as generalized additive models (GAM) relaxing the linearity assumption.

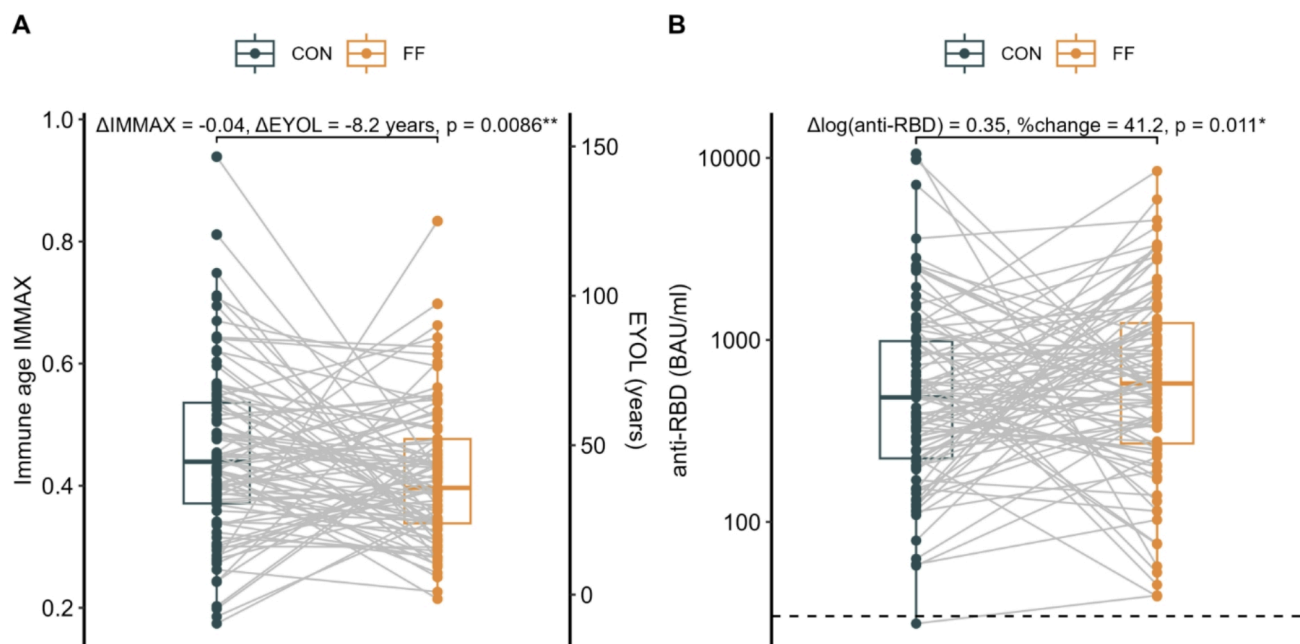


Figure 6

Firefighters (FF, N=91) are immunologically younger (A) and have higher titers of anti-RBD antibodies (B), than controls (CON, N=91) from the general population matched for age, sex, vaccine and vaccination time lag as well as for smoking habits and health status. The dashed horizontal line refers to the lowest effective titer of 30.3 BAU/ml. Statistical comparison by paired t-tests performed after propensity score matching.

Supplementary Files

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