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Research paper

Initial observations on age, gender, BMI and hypertension in antibody responses to SARS-CoV-2 BNT162b2 vaccine

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ABSTRACT

Background: Literature data suggests that age, gender and body mass index (BMI) could be associated with difference in immune responses to vaccines. The first goal of the study was to analyze the antibody titre seven days after the second dose of BNT162b2 vaccine in a group of 248 healthcare workers (HCWs). The second goal was to analyze how antibody titre changes in correlation with age, gender, BMI and hypertension.
Methods: An immunogenicity evaluation was carried out among HCWs vaccinated at the Istituti Fisioterapici Ospitalieri (IFO), Rome, Italy. All HCWs were asked to be vaccinated by the Italian national vaccine campaign at the beginning of 2021. 260 vaccinated HCWs were enrolled in the study. All eligible participants were assigned to receive the priming dose in two weeks' time and the booster dose exactly 21 days thereafter. Blood and nasopharyngeal swabs were collected at baseline and 7 days after second dose of vaccine. Quantitative measurements of IgG antibodies against S1/S2 antigens of SARS-CoV-2 were performed with a commercial chemiluminescent immunoassay. Presence of SARS-Cov-2 in nasopharyngeal swab was determined by commercial RT-PCR testing.
Findings: 248 HWCs were analyzed, 158 women (63.7%) and 90 men (36.3%). After the second dose of BNT162b2 vaccine, 99.5% of participants developed a humoral immune response. The geometric mean concentration of antibodies among the vaccinated subjects after booster dose (285.9 AU/mL 95% CI: 249.5−327.7) was higher than that of human convalescent sera (39.4 AU/mL, 95% CI: 33.1−46.9), with $p<0.0001$. Multivariate linear regression analysis of AU/mL by age, gender and BMI multivariate was performed by the inclusion of covariates. This analysis demonstrated that age ($p<0.0001$) and gender ($p = 0.038$) are statistically associated with differences in antibody response after vaccination, whereas BMI and hypertension have no statistically significant association ($p = 0.078$ and $p = 0.52$ respectively).

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Interpretation: 99.5% of HCW developed a humoral immune response and female and young participants seem to have an increased capacity to mount humoral immune responses. BMI and hypertension seem not associated with difference in immune response to the vaccine.
Funding: None.

Research in context

Evidence before this study

A large phase 2/3 clinical trial with 44,000 people showed that a two-dose regimen of the vaccine BNT162b2, developed by BioNTech and Pfizer, has 95% efficacy in preventing symptomatic COVID-19.

More data are certainly needed to assess the immunogenic efficacy and their correlation with age, gender and BMI.

Added value of this study

99.5% of HCW developed a humoral immune response and female and young participants seem to have an increased capacity to mount humoral immune responses. On the other side, BMI and hypertension seems not associated with difference in immune response to the vaccine.

Implications of all the available evidence

This report marks an important point in the current context, both for vaccine efficacy and for public health measures. Although further studies are needed, this data may have important implications to the development of tailored vaccination strategies for COVID-19.

1. Introduction

Since the first cases of COVID-19 were described in December, a health emergency with major social and economic disruptions has spread worldwide.

The World Health Organization, on the 11th of March 2020, announced the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak a pandemic.

Although rigorously applied, control measures such as the use of masks, physical distancing and contact tracing, helped to limit viral transmission; however no substantial benefits were noted, and it soon became clear that vaccines represented the main viable road to get out of the pandemic.

Since the genetic sequence of SARS-CoV-2 on January 11, 2020, scientists and biopharmaceutical manufacturers focused their research on developing a vaccine.

Currently, there are over 238 vaccine candidates being developed against COVID-19, with 63 at various stages of human clinical trial test [1].

A large clinical trial phase 2/3 with 44,000 people showed that a two-dose regimen of the vaccine BNT162b2, developed by BioNTech and Pfizer, has 95% efficacy in preventing symptomatic COVID-19. The same study showed that safety over a median of 2 months was similar to that of other viral vaccines [2].

As a consequence of these results, on December 11th, 2020, the U. S. Food and Drug Administration authorized vaccine BNT162b2 for emergency use. This was soon followed by the European Medicines Agency on 21 December 2020.

Nonetheless, the efficacy of protection from infection of BNT162b2 vaccine has not been established.

To overcome this limit, antibody titre can be used to predict protection against SARS-CoV-2, as already done for many viruses in humans and for SARS-CoV-2 in animal challenges [3, 4].

Two studies by BioNTech/ Pfizer's presented immunogenicity data: preliminary results are encouraging, but antibody responses with a double 30 micrograms regimen is reported only in 22 patients [5, 6].

More data is certainly needed to assess the efficacy and thus protection against the virus.

In this setting, we report the early experience with BNT162b2 vaccination in a medical population. The first goal of our study was to analyze the antibody titre response 7 days after the second dose of vaccine in a group of 248 healthcare workers (HCW). Our second goal was to analyze how the antibody titre changes in correlation with age, gender and BMI.

2. Methods

2.1. Study design and participants

A collaborative team carried out an immunogenicity evaluation among HCWs vaccinated at the Istituti Fisioterapici Ospitalieri (IFO). All HCWs were asked to be vaccine by the national vaccine campaign at beginning 2021.

First 260 vaccinated subjects were enrolled in the study and gave written consensus to the study. Twelve HCW were excluded according to inclusion criteria.

All eligible participants were assigned to receive the priming dose in two weeks' time and the booster dose exactly 21 days thereafter.

The study protocol complied with the tenets of the Helsinki declaration and was approved by the institutional scientific ethics committee (protocol RS1463/21) and registered to a Clinical Trial registry ISRCTN55371988. Participants were requested to provide written informed consent.

All the enrolled participants met the following inclusion criteria: 1) provided written informed consent 2) age between 18 and 75 years, 3) health workers employed at the Istituti Fisioterapici Ospitalieri (IFO), 4) vaccinated at the Istituti Fisioterapici Ospitalieri (IFO).

Key exclusion criteria included: 1) evidence of current or previous SARS-CoV-2 infection by either anamnesis, serological or microbiological test through nasopharyngeal swab before enrolment, 2) treatment with immunosuppressive therapy, 3) immunosuppression-associated pathology, and 4) pregnancy.

Human SARS-CoV-2 infection convalescent sera (n = 59) were drawn from HCW donors (mean age 45) at least 14 days after PCR-confirmed diagnosis and at a time when the participants were asymptomatic. The donors were divided into groups based on symptoms: symptomatic infections (n:41); asymptomatic infections (n:18).

The manufacturer's (BioNTech/Pfizer, USA) instructions for storage and administration of vaccine were followed.

The COVID-19 mRNA Vaccine BNT162b2 was stored in an ultra-low temperature freezer at −80 °C. The undiluted vaccine was stored for up to 2 h at temperatures up to 25 °C, prior to use.

The mRNA vaccine was administered as a 30 microgram / 0.3 ml intramuscular injection into the deltoid muscle on days 1 and 22 of the study.

We used a questionnaire to collect data on the participants' socio-demographic and health characteristics. Participants were stratified by age, sex, body mass index (BMI) and hypertension.

Participants had a nasopharyngeal swab for SARS-CoV-2 RT-PCR testing (Viracor, Eurofins Clinical Diagnostics, U.S.A.), and were assessed for the presence of SARS-CoV-2–binding antibodies (The LIAISON® SARS-CoV-2 S1/S2 IgG, test Diasorin, Italy) and at baseline and 7 days after BNT162b2 booster dose.

We decided to use 7day post booster dose for two main reasons: i) to reveal how early was the vaccine response; ii) efficacy of BNT162b2 was analyzed with a 7 days starting point after the second dose.

2.2. Assessment of SARS-CoV-2 in nasopharyngeal swab

A nasopharyngeal swab was collected by standard procedures and presence of SARS-CoV-2 was determined by RT-PCR testing (Viracor, Eurofins Clinical Diagnostics, U.S.A.) following the manufacturer's instruction.

2.3. Assessment of SARS-CoV-2 binding antibody

Peripheral venous blood samples of 7–8 mLs were obtained, serum collected and stored at +4 °C.

Quantitative measurement of IgG antibodies against S1/S2 antigens of SARS-CoV-2 was performed with a commercial chemiluminescent immunoassay (The LIAISON® SARS-CoV-2 S1/S2 IgG, test Diasorin, Italy) according to the manufacturer's instruction.

2.4. Statistical analysis

Geometric Mean of AU/mL and its 95% confidence interval was reported for the entire series and for each subgroup. A generalized linear model using the logarithm of titer as dependent variable was implemented to assess correlation between gender, age and BMI with serum concentration.

Age was then categorized through quartiles and BMI subgroups were created according to WHO classes. A oneway ANOVA was used to assess differences between groups, with post-hoc evaluation of differences among subgroups adjusted for multiplicity by Tukey.

A sensitivity analysis by means of a non-parametric test (Kruskal-Wallis) was performed to take into account the presence of some outlier which could have influenced ANOVA results.

Statistical analysis was done using SPSS Statistics software version 21. A $p < 0.05$ was considered statistically significant.

2.4.1. Role of the funding source

The study did not receive any external financial support. All authors had full access to the full data in the study and accepted responsibility to submit for publication.

3. RESULTS

Among 260 HCWs who gave written consensus to the study, 12 HCW were excluded according to inclusion criteria.

248 HWCs were analyzed, 158 women (63.7%) and 90 men (36.3%). All participants were of Caucasian ethnicity and median age was 47 years, (range 23–69) (Table 1). Nasopharyngeal swab test at baseline and seven days after booster dose did not reveal presence of SARS-CoV-2 in any of the participants. After second dose of BNT162b2, 99.5% of participants developed a humoral immune response respect to the baseline (T0) serum level. Only one participant was a non-responder after the second dose. Seven days after the booster dose, S1-S2 binding antibody concentration was in the range of 3.8–2460 AU/mL. The antibody geometric mean concentration (AbGMC) among vaccinated subjects (285.9 AU/mL 95% CI: 249.5–327.7) was higher than that of human convalescent sera (39.4 AU/mL, 95% CI: 33.1–46.9), with $p<0.0001$ (Fig. 1).

Table 1
GMC and 95% CI by age, gender, BMI and hypertension at T0 and T1 (7 days after BNT162b2 booster dose).

Characteristic	Number	GMC (95% CI)	GMC (95% CI)
Sampling		**T0**	**T1**
Age			
<=37	62	4.0 (3.9–4.1)	453.45 (363.6–565.5)
37–47	63	4.3 (4.0–4.6)	330.91 (253.7–431.6)
47–56	64	4.1 (3.9–4.4)	239.77 (182.4–315.1)
>56	59	4.0 (3.9–4.2)	182.40 (137.8–241.4)
Gender			
Female	158	4.0 (3.9–4.2)	338.49 (286.3–400.2)
Male	90	4.2 (4.0–4.5)	212.63 (170.2–265.6)
BMI			
Under-weight	19	3.9 (3.7–4.1)	455.41 (311.5–665.7)
Normal-weight	147	4.2 (4.0–4.3)	325.84 (277.9–382.0)
Pre-obesity	56	4.0 (3.9–4.2)	222.40 (168.9–292.9)
Obesity	26	4.2 (3.8–4.7)	167.05 (90.2–309.3)
Hypertension			
No	217	4.1 (4.0–4.2)	307.42 (267.4–353.4)
Yes	31	4.3 (3.9–4.7)	172.18 (109.0–272.1)

GMC: geometric mean concentration; CI: confidence interval; BMI: body mass index according to Weir CB and Jan A. Paired tests between T0 and T1 were significant for all the variable ($p<0.0001$).

Multivariate analysis accounting for potential confounding was performed by the inclusion of covariates. Data on multivariate linear regression of AU/mL is reported in Table 2. This analysis demonstrated that age ($p<0.0001$) and gender ($p = 0.038$) are statistically associated with differences in antibody response after vaccination, whereas BMI and hypertension have no statistically significant association ($p = 0.078$ and $p = 0.52$ respectively).

Then, four age quartiles, gender and BMI classes were exanimated using a multi-group analysis.

The antibody titre was greater in younger participants compared to older participants with statistically significant differences: <=37 vs 47–56 $p = 0.005$, <=37 vs >56 $p<0.0001$, 37–47 vs >56 $p = 0.01$ (Table 1 and Fig. 2).

Antibody responses of greater magnitude was shown in women vs in men; this difference (338.5 AU/mL vs 212.6 AU/mL) was statistically significant with $p = 0.001$ (Table 1 and Fig. 2). Differences among BMI classes and antibody titres was noticed ($p = 0.02$). Humoral response was observed in all the study participants, with higher values in under- and normal-weight groups compared to pre-obesity and obesity groups. After adjusting for age and gender p was 0.04

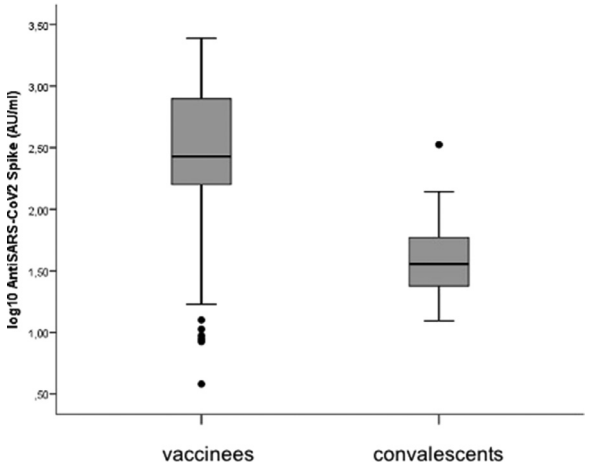

Fig. 1. Anti SARS-CoV-2 IgG in vaccinated HCW vs COVID-19 convalescent sera.
Sera from vaccinees and convalescents were collected as in methods. Antibody levels were expressed as log10 of concentration in Arbitrary Unit (AU).

Table 2
Multivariate linear regression of AU/mL of age, gender and BMI.

	Beta (95% CI)	P value
AGE (in years)	−0.023 (−0.035; −0.011)	<0.0001
BMI (kg/cm^2)	−0.028 (−0.060; +0.003)	0.078
GENDER (male vs female)	−0.290 (−0.564; −0.016)	0.038
HYPERTENSION (yes vs no)	−0.135 (−0.549; 0.279)	0.52

considering four age classes, and p was 0.068 considering age as linear data. Hypertension was associated with lower AbGMC titre with $p = 0.006$, but this association was not confirmed after adjusting for age ($p = 0.22$). Kruskal-Wallis non-parametric test, applied to data on Fig. 2, was utilized to assess differences in distributions among all considered variables as a sensitivity analysis. Non-parametric analysis gave rise to significant p values, namely: $p<0.0001$, $p = 0.033$, $p<0.0001$ and $p = 0.006$ for age (4 classes), BMI (4 classes), gender and high blood pressure, respectively.

4. Discussion

During the last year we have witnessed a remarkable effort of researchers and the pharmaceutical industry in the development of a vaccine against SARS-CoV-2.

With this paper, we present an independent study on antibody titre against S1/S2 SARS-CoV-2 in HCWs 7 days after the second dose of BNT162b2: >99% of participants demonstrated antigen-specific humoral response respect to baseline level and no one showed positive nasopharyngeal test during the study. Only one participant was a non-responder after the second dose. However, we don't have any valid explication for this data.

Although the role of neutralizing antibodies to SARS-CoV-2 is under investigation, measurement of serum neutralizing activity has been demonstrated to correlate with protection for other respiratory viruses, such as influenza [3] or respiratory syncytial virus [7] and is commonly accepted to be a functional biomarker of in vivo disease protection [8]. In this study we utilized a chemiluminescent immunoassay that detect S1/S2 specific antibodies, even though it was not specifically designed to detect neutralizing antibodies.

However, manufacturer indicates that with 80-AU/ml levels, the probabilities of having plaque reduction neutralization titres of 1:80 and 1:160 was 92% and 87%, respectively [9]. Thus, we can assume that at least a consistent part (93.2%) of the enrolled population showing >80AU/mL should have developed neutralizing antibodies.

The principal limitations of the study are the following. Our study is a single center study, and it was with a limited number of participants, in addition, all subjects of Caucasian ethnicity, therefore it cannot be assumed representative of general population nor of non-Caucasian population. Furthermore, we used a questionnaire to collect data on the participants' socio-demographic and health characteristics, and the possibility of self-reporting bias should be considered. No comorbidities other than hypertension were collected from the self-administrated questionnaire.

We analysed first 260 vaccinated HCWs and this could be a responder bias.

Literature data suggests that age, gender and BMI could be associated with difference in immune responses to vaccines. Therefore, the impact of age, gender and BMI were analyzed in our cohort of HCWs.

It is well known that aging and related immunosenescence may lead to poor immune response to vaccine [10]. In particular qualitative differences were observed in the memory B cells and plasma compartment in older adults. This included class switch recombination and differentiation in to plasmacells [11, 12]. Additional qualitative change with age is the expansion of a pro-inflammatory subset of B cell similar to age-associated B cells (ABCs) described in mice [13, 14]. It was shown that increased level of$CD27^+$ABCs in blood of the elderly was associated with reduced titre of influenza specifical antibodies [15]. Our data evidenced statically significance differences by age in humoral response to vaccine.

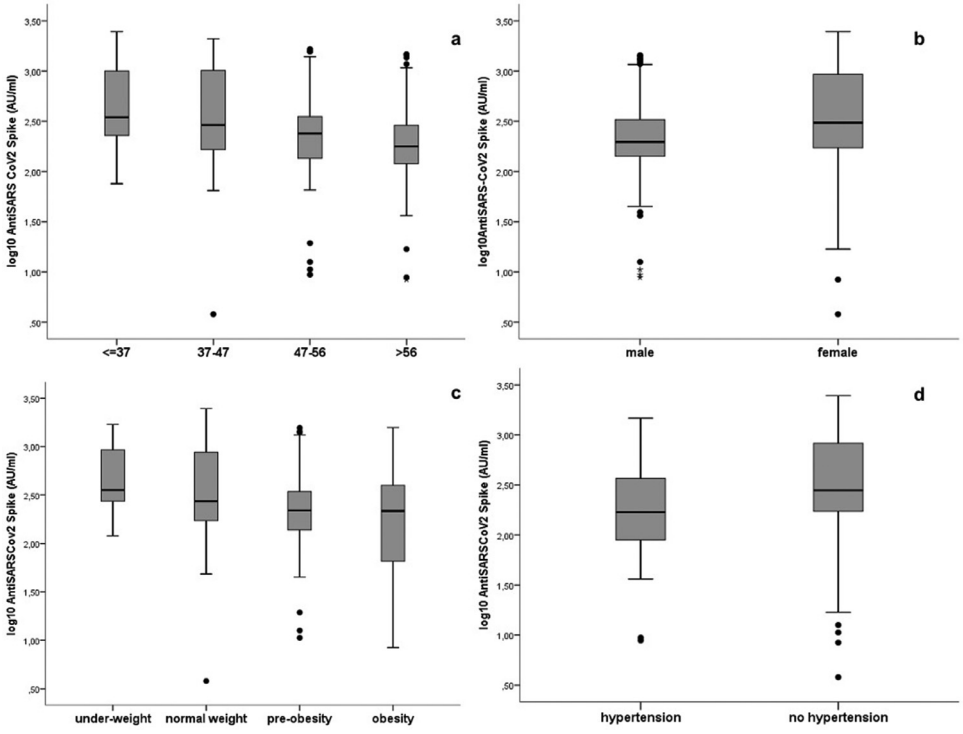

Fig. 2. Levels of anti SARS-CoV-2 IgG by age (a), gender (b), BMI (c) and hypertension (d).
Seven days after booster dose, serum was collected from all participants. Antibody levels were expressed as log10 of concentration in Arbitrary Unit (AU). Age was categorized according to quartiles. Body mass index (BMI) classes were categorized according to Weir CB & Jan A (32). Data on hypertension was collected from self-administered questionnaire.

Evidence from a recent meta-analysis suggest that COVID-19 exhibits differences in morbidity and mortality between sexes. Male patients have almost three times the odds of necessitating intensive treatment unit admission and higher odds of death compared to females [16]. Our results also confirmed this difference in vaccine response. Women produce higher antibody titres in response to the trivalent inactivated seasonal influenza vaccination (TIV) [17, 18], as well as to most other pathogen vaccines [19]. More specifically, females achieve equivalent protective antibody titres to males at half the dose of TIV [20], with serum testosterone levels inversely correlating with TIV antibody titres [21].

The effectiveness of COVID-19 vaccines in people with obesity is a critical issue. Since obesity is a major risk factor for morbidity and mortality for patients with COVID-19 [22], it is mandatory to plan an efficient vaccination program in this subgroup [23, 24].

The constant state of low-grade inflammation, present in overweight people, can weaken some immune responses, including those launched by T cells, which can directly kill infected cells [25]. Obesity was associated with decreased activation of $CD8^+$ *T* cells, stimulated with live influenza virus [26].

Moreover, vaccines against influenza [27], hepatitis B [28], rabies [29] and tetanus [30] have shown reduced responses in those who have obesity compared with those who are lean. A number of studies demonstrated BMI association with decreased serum response to vaccine during influenza vaccine season [26, 31]. Our data did not confirm this association for BNT162b2 vaccine.

To our best knowledge, no literature data exists on association of hypertension with poor vaccine response. However, statistical significance of our data was not confirmed by age matching analysis suggesting bias on this data.

Taken together, collected data shows immune response in 99,5% with a correlation with age and gender, as higher antibody titre was detected in younger people and in females.

Although further studies are needed, this data may have important implications to the development of tailored vaccination strategies for COVID-19.

At the same time, we strongly believe that our results are extremely encouraging and useful for the scientific community.

Data sharing statement

Data is going to be added to the clinical trial registry (ISRCTN55371988) where they will be available

Declaration of competing interest

All authors declare no conflict of interest to disclose.

CRediT authorship contribution statement

Raul Pellini: Conceptualization, Writing – review & editing, Supervision. **Aldo Venuti:** Conceptualization, Writing – original draft. **Fulvia Pimpinelli:** Methodology. **Elva Abril:** Data curtion. **Giovanni Blandino:** Data curtion. **Flaminia Campo:** Writing – original draft. **Laura Conti:** Data curtion. **Armando De Virgilio:** Data curtion. **Federico De Marco:** Data curtion. **Enea Gino Di Domenico:** Investigation. **Ornella Di Bella:** Investigation. **Simona Di Martino:** Investigation. **Fabrizio Ensoli:** Methodology. **Diana Giannarelli:** Writing – original draft. **Chiara Mandoj:** Investigation. **Valentina Manciocco:** Data curtion. **Paolo Marchesi:** Data curtion. **Francesco Mazzola:** Data curtion. **Silvia Moretto:** Data curtion. **Gerardo Petruzzi:** Data curtion. **Fabrizio Petrone:** Investigation. **Barbara Pichi:** Investigation. **Martina Pontone:** Methodology. **Jacopo Zocchi:** Investigation. **Antonello Vidiri:** Investigation. **Branka Vujovic:** Writing – original draft, Investigation. **Giulia Piaggio:** Data curtion. **Aldo Morrone:** Conceptualization. **Gennaro Ciliberto:** Conceptualization, Writing – review & editing.

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