SARS-CoV-2 anti-spike antibody titres after vaccination with BNT162b2 in naïve and previously infected individuals

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ABSTRACT

Great expectations are placed in vaccines against COVID-19 to control the pandemic. We reviewed the antibody titres in a cohort of healthcare workers (HCWs) vaccinated with BNT162b2 to assess the influence of a previous infection on them. We stratified the results according to the individual history of nasopharyngeal swab (NPS) and symptoms. Among 3475 HCWs the highest titres were recorded among those infected more than 6 months before vaccination, independently of symptoms, followed by those infected less than 6 months before vaccination, especially in those with symptoms, and by uninfected HCWs. Vaccination with BNT162b2 can boost immunity acquired through infection, particularly in those infected more than 6 months before vaccination.

Introduction

The pandemic of Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2), is hardly affecting the entire world, with 155,373,584 cases and 3,245,391 related deaths as of 1th of May 2021 and great expectations are placed in mass vaccination. Currently, the four COVID-19 vaccines approved in the European Union are the mRNA-based BNT162b2 and mRNA-1273 and the adenoviral vector-based ChAdOx1 nCoV-19 and Ad26.COV2-S [1]. All these vaccines are designed to elicit an immune response directed toward the S1 spike protein of SARS-CoV-2 [2]. Both BNT162b2 and mRNA-1273 in the

first 100 days after vaccination were able to elicit specific antibodies titres and neutralizing antibodies concentrations above those observed among COVID-19 human convalescent serum [3]. Uncertainties remain regarding the impact of vaccination of previously infected individuals, with preliminary data showing higher antibody titres in those who were infected [4–7]. Moreover, the severity of COVID-19 has been directly correlated with the persistence of detectable neutralizing antibodies in serum [8].

Methods

We assessed the anti-S1 antibodies titres (Elecsys Anti-SARS-COV-2 S, Roche Diagnostics, Monza, Italy) on 3475 healthcare workers (HCWs) of the IRCCS Ospedale Maggiore Policlinico of Milan, Italy, 28 days after having received the second dose of BNT162b2 vaccine (data as of May 1). All the HCWs received two doses of BNT162b2 vaccine, irrespective of previous SARS-COV-2

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Table 1
Association between selected variables and anti-S1 antibody titres among healthcare workers in a research and teaching hospital in Milan, Italy.

Variable	N subjects	Anti-S (U/mL) Median	Anti-S>7500 U/mL[*] N	%	RR	95% CI
All	3475	1577	530	15.2		
Gender						
Women	2475	1602	358	14.5	1.00	Reference
Men	1000	1501	172	17.2	1.00	0.87−1.15
p-Value[**]		*0.10*		*0.12*		
Age (years)						
<35	1162	1976	194	16.7	1.00	Reference
35−44	669	1553	88	13.1	0.67	0.55−0.82
45 + 54	903	1449	134	14.8	0.72	0.60−0.86
55+	741	1208	114	15.4	1.00	0.66−0.94
p-Value[**]		*0.0001*		*0.22*		
BMI (kg/m^2)						
<20	602	1487	75	12.5	1.00	Reference
20−24.99	1612	1556	229	14.2	1.00	0.80−1.24
25−29.99	765	1556	127	16.6	1.20	0.95−1.54
30+	266	2029	60	22.6	1.34	1.02−1.76
p-Value[**]		*0.004*		*0.006*		
Cigarette smoking						
Never	1908	1756	342	17.9	1.00	Reference
Former	567	1514	83	14.6	0.85	0.71−1.02
Current	804	1218	71	8.8	0.63	0.51−0.77
p-Value[**]		*0.0001*		*<0.001*		
Nasopharyngeal swab/symptoms						
Never positive	2968	1374	200	6.7	1.00	Reference
Positive <180 days before/No	70	2589	18	25.7	3.67	2.27−5.93
Positive <180 days before/Yes	271	7500	161	59.4	9.00	7.55−10.7
Positive 180+ days before/No	14	7500	12	85.7	11.5	8.1−16.4
Positive 180+ days before/Yes	152	7500	139	91.4	13.3	11.3−15.7
p-Value[**]		*0.0001*		*<0.001*		

Abbreviations: BMI, body mass index; CI, confidence interval; LOQ, limit of quantification; RR, risk ratio.
RR were calculated with a multivariable Poisson regression model with robust standard error containing all the variables in the table.
  [*] Higher measurement limit: 7500 U/mL.
  [**] p-Values calculated with Kruskal–Wallis (quantitative titres) or chi-squared (titres > LOQ) tests.

infection, 21 days apart. We reviewed the results of SARS-COV-2 RT-PCR on nasopharyngeal swabs (NPSs) (AllplexTM2019-nCoV Assay, Seegene, Seoul, South Korea) performed for active surveillance, presence of symptoms or contact with COVID-19 case, by the vaccinated HCWs since the beginning of the COVID-19 pandemic. We examined the association of anti-S1 titres with gender, age, BMI, smoking, and a five-category variable representing the combination of NPS results and symptoms, i.e., never positive, positive less than 180 days before Ig testing (without or with symptoms), positive more than 180 days before (without or with symptoms). Those infected less than 180 days before Ig testing were defined as recently infected whereas those receiving immunosuppressive drugs for transplantation or autoimmune disorders were classified as with immune deficiencies. We used Kruskal–Wallis test to analyse quantitative titres and chi-squared test to analyse above the higher measurement limit (HML) of the method (7500 U/mL). Finally, we fitted a multivariable Poisson regression model with robust standard error containing all these variables to calculate adjusted risk ratios (RR) and 95% confidence intervals (CI) of high (i.e., >HML) vs low titres. Analyses were performed with Stata 16 (StataCorp. 2019) [9]. The study was exempted from a formal approval by the internal review board of our institution considering that the results are aggregate data collected for health surveillance purposes.

Results

Out of 3475 subjects, only six (0.17%) had non-detectable anti-S1 (i.e., less than the limit of quantification of <0.4 U/mL), four of them reported underlying immune deficiencies. Median anti-S1 titres (Table 1) were associated with age (negatively) and BMI (positively). Smokers showed lower median titres than never smokers.

Subjects who never had positive NPS tests had the lowest median titres. Asymptomatic subjects infected less than six months before Ig testing had lower median titres than recently infected symptomatic ones and those infected more than six months before. When analysing crude proportions of high (>HML) titres, the associations with BMI and smoking were confirmed. Moreover, we found a positive trend with NPS results and symptoms: 6.7% had high anti-S1 among never positives, 25.7% among asymptomatic recently infected, 59.4% among symptomatic recently infected, and about 90% (irrespective of symptoms) in those infected more than six months before. The multivariable analysis confirmed that subjects aged <35 years, overweight/obese, and never smokers had higher frequency of high (>HML) titres. Also confirmed was the pattern according to NPS results and presence of symptoms.

Discussion

Our serologic data suggest that the complete vaccination schedule with BNT162b2 elicit a vigorous immune response, assessed in terms of raw anti-S1 antibody titres, in both uninfected and previously exposed individuals. This response appears higher in those who were infected more than 6 months before vaccination than those infected more recently. Interestingly, the highest antibody titres were found among those aged <35 years, those overweight/obese, and who never smoked. Among those infected, antibody tires above the HML were associated with the presence of symptoms.

The detection of higher antibody titres among subject previously infected is an expected finding, with the vaccination acting as a booster of naturally occurring immunity. Overall, our data are in accordance with preliminary reports already available [4–7]. Several reasons can justify the higher antibodies titres observed in the

NPS+ ≥180 group. On one hand, they can be the consequence of multiple exposures to SARS-CoV-2 occurred after the first infection, which acted as natural boosters of the immune response. On the other hand, it is well known how high recall and anamnestic responses to vaccination are associated with intervals of at least 3–4 months between stimuli, with longer intervals associated with generally greater responses. On the basis of our results, previously infected individuals can be vaccinated with the reassurance of achieving elevated antibody titres.

Regarding the highest titres observed among those who presented symptoms, this parallel similar observations that our and other groups have made also for anti-N antibodies, elicited only by natural infection [10]. Among the other, Legros et al. demonstrated how neutralizing antibodies (nAb) titres correlated strongly with disease severity and with anti-spike IgG levels, with patients from intensive care units exhibiting high nAb titres whereas asymptomatic or exclusive outpatient-care patients had no or low nAbs [11]. It remains to be understood if vaccination can elicit nAbs in previously infected individuals in the same way that anti-S1 antibodies are stimulated.

Interestingly, as we previously observed for anti-N antibodies, anti-S1 antibodies titres were lower among smokers [10]. This confirm the impact of smoking on the immune system and its ability to mount an humoral response to inhaled but also injected antigens, a finding in contrast with previous evidence which suggested an influence only on inhaled antigens [12]. Cumulatively, smokers are particularly at risk during COVID-19 pandemic for both the increased probability of having respiratory manifestations and the lower ability of mounting an effective immune response.

Intriguingly is the positive association found between the anti-S1 antibody titres and the BMI. Indeed, SARS-CoV-2 IgG antibodies have been negatively associated with BMI in COVID-19 obese patients [13]. This is in accordance with what has been observed after influenza infection or influenza vaccination, which has been linked to the higher inflammation, the reduced number of suppressive T-regulatory cells and the decreased populations of bone marrow–resident B cells observed among obese individuals [14,15]. After influenza vaccination in obese patients, BMI was correlated positively with higher initial fold increase in IgG antibodies but, 12 months post vaccination, higher BMI was associated with a greater decline in influenza antibody titres. A similar trend could be expected in our cohort and a long term follow-up of antibody titres is needed to verify this hypothesis.

Several questions remain unanswered: the duration of the antibody response, the cellular immune response elicited, the minimum titre required to be protected from COVID-19, the effectiveness of vaccination against SARS-CoV-2 variants and the prevalence of neutralizing antibodies among the total value of antibodies. Overall, vaccination with BNT162b2 elicited strong antibody responses in both previously infected and uninfected individuals and this response was particularly relevant among those recently infected who reported symptoms and in those with remote infection, irrespective of symptoms.

Contributors

AL, DC and AG conceived the study. PB, SUR, DC, FC, MO collected the data. DC performed statistical analysis. AL wrote the first draft of the manuscript. All the authors reviewed the final version of the manuscript.

Competing interests

None declared.

Ethical approval

Not required.

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