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Immunogenicity of BNT162b2 mRNA-based vaccine against SARS-CoV-2 in people with cystic fibrosis

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Short title: Immunogenicity of BNT162b2 in CF.

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Abstract

During the SARS-CoV-2 vaccination campaign, people with CF (pwCF) were considered a clinically vulnerable population. However, data on immunogenicity of anti-SARS-CoV-2 vaccines in pwCF are lacking. We conducted a prospective study enrolling all patients aged >12 and followed-up in our CF centre, who received two doses of the BNT162b2 vaccine in March-October 2021. They underwent a blood sample for quantification of antibodies to the SARS-CoV-2 spike protein receptor binding domain immediately before receiving the first dose and after 3 and 6 months from the second dose. We enrolled 143 patients (median age: 21 years, range: 13-38); of whom 16 had a previous infection. Median antibody titer (interquartile range) after 3 months from vaccination was 1288 U/mL (730-2115) and decreased to 918 U/mL (534-1488) after 6 months (P<0.0001). Median values were higher among previously infected patients as compared to those naïve to SARS-CoV-2 (9107 *vs* 1229 U/mL at 3 months and 4810 *vs* 829 U/mL at 6 months, P<0.0001) with no significant differences in the rate of decline over time (P=0.135). All pwCF mounted an antibody response after two-doses of BNT162b2 vaccine that waned at 6 months from vaccination. Age ≥30 years and use of inhaled corticosteroids were associated with a lower humoral response.

Keywords: cystic fibrosis; SARS-CoV-2; Covid-19, vaccine; antibody response; humoral response.

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Background

Cystic fibrosis (CF) is the most frequent life-threatening genetic disease among Caucasians caused by mutations in the CF Transmembrane Conductance Regulator (CFTR) gene. The gene codes the CFTR protein, an ion channel that regulates chloride and bicarbonate traffic at the cell surface and its abnormal function causes production of thick secretions in many organs, including the pancreas and the lungs. Clinical manifestations of CF mainly include fat malabsorbtion, requiring pancreatic enzyme replacement therapy, and frequent respiratory infections leading to lung damage and eventually progression to end-stage lung disease and need of lung transplantation [1].

People with CF (pwCF) are considered a clinically vulnerable population and thus they were vaccinated at the very beginning of the vaccination campaign in many countries, including Italy (February-March 2021). However, data on how this population responded to the vaccination are limited to a small study on 33 pwCF showing higher antibody responses as compared to a group of healthy subjects [2].

A potential defect in the immune response to influenza vaccine was suggested during the 2009/H1N1 pandemic [3], which may be linked to the defective expression of CFTR in lymphocytes [4,5]. In addition, in this population, innate and adaptive immunity is dysregulated due to inherited and acquired factors, including epithelial barrier function, pathogen sensing, leukocyte and phagocyte recruitment and communication between innate and adaptive immunity [6,7]

Moreover, the maintenance therapy of pwCF may involve the administration of several inhaled and systemic drugs that may affect the immunological response to vaccines. Among these drugs, steroids are frequently prescribed to reduce lung inflammation and to treat wheezing; pwCF also receive frequent antibiotic courses to eradicate gram-negative bacteria from the respiratory airways. Antibiotics affects gut microbiome and microbiome alteration has been linked to reduced immunogenicity and efficacy of vaccines [8,9].

Thus, this study aims to evaluate the antibody response to BNT162b2 mRNA-based vaccine against SARS-CoV-2 in pwCF and to characterize subgroups of this population with low responses.

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Materials and Methods

All patients aged >12 years, in regular follow-up at the Reference Centre for CF of Lombardia region who received two doses of the mRNA-based vaccines BNT162b2 (COMIRNATY BioNTech Manufacturing GmbH) between March and October 2021 were included in this prospective study. They underwent 3 blood samples for quantification of antibodies to the SARS-CoV-2 spike protein receptor binding domain (S1-RBD). Serum titers were measured immediately before administering the first dose of the vaccine and at 3 and 6 months after receiving the second dose using an electrochemiluminescence immunoassay (Elecsys Anti-SARS-CoV-2 S Roche Diagnostics, Monza, Italy) (positive cutoff: 0.8, lower limit of quantification, LLOQ: 0.4 U/mL, upper limit of quantification, ULOQ: 12,500 U/mL, sensitivity: 98.8% and specificity: 100%). Values below the LLOQ were set to LLOQ/2, and values above the ULOQ were set to ULOQ. This serological assay uses a recombinant protein representing the S-RBD protein in a one-step double antigen sandwich assay format. Samples were incubated with a mix of biotinylated and ruthenylated RBD antigen and double-antigen sandwich immune complexes are formed when corresponding antibodies are present. After addition of streptavidin-coated microparticles, the DAGS complexes bind to the solid phase via interaction of biotin and streptavidin. The microparticles are magnetically captured on the surface of the electrode and electrochemiluminescence is induced by applying a voltage and measured with a photomultiplier. Samples with a value ≥0.8 U/mL were considered “reactive” (positive). The analyses were performed at the Clinical Laboratory of Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Milan, Italy.

Information on prior infection by SARS-CoV-2, including positive reverse transcriptase-polymerase chain reaction (RT-PCR) molecular test and occurrence of Covid-19 symptoms was collected through a telephone interview carried out by the attending physicians, while clinical data and microbiological results of sputum cultures were retrieved from patient medical records. During the interview a diary card recording solicited local and systemic adverse reactions were also completed

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for 7 days after each administration. Severity of adverse reactions was graded using the following criteria: mild (transient or mild discomfort for <48 hours, no interference with activity, and no medical intervention or therapy required), moderate (mild-to-moderate limitation in activity, and no or minimal medical intervention or therapy required), severe (substantial limitation in activity and medical intervention or therapy required), or potentially life-threatening (requiring assessment in emergency department or admission to hospital) [3].

In patients naïve to SARS-CoV-2, antibody titers were compared across groups defined by demographic characteristics (sex and age), indicators of disease severity (pancreatic insufficiency, underweight, infection by P. *aeruginosa*) and current CF maintenance treatment, including inhaled and systemic antibiotics, azithromycin, inhaled corticosteroids (ICS) and CFTR modulators. Humoral response was also compared among patients reporting severe adverse reactions *vs* those who had none or only mild reactions and among patients with systemic *vs* local reactions after the administration of the vaccine.

Exocrine pancreatic status was determined according to faecal elastase-1 levels with level <200 µg/g of faeces being indicative of pancreatic insufficiency. Patients were considered underweight if their z-score of BMI-for-age was < -1.64 (i.e. < 5^{th} percentile) (for patients aged ≤20 years) or their BMI was <18.5 kg/m^2 (for older patients) [10]. Z-scores of BMI were obtained using the Italian reference data [11]. P. *aeruginosa* infection was defined by a positive sputum culture during the last visit preceding vaccination.

Given the skewed distribution of the antibody titers they were log10 transformed before analysis. Antibody titers at 3 and 6 months post-vaccination and their variations were compared across patient groups using linear mixed effect models with subject random intercept. The model included the antibody titer as dependent variable and group, time (3 or 6 months) and their interaction as fixed effects. The statistical significance of the fixed effects was evaluated using the likelihood ratio test. The significance of the group effect indicates differences between groups in antibody titers irrespective of the time of the measurement, while the significance of the time effect indicates

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difference between the two time points (i.e. 3 *vs* 6 months post-vaccination), and the interaction denotes between-group differences in the variations over time. To test the independent associations between explanatory variables and antibody response, we fitted a multivariable linear mixed effect model including all the factors significantly associated with antibody response in the analysis described above. The estimated β coefficients and the 95% confidence intervals were used to evaluate the effect of each factor on the log10 antibody titer.

All tests were two-sided with significance level set at 0.05.

Results

Table 1 summarizes the main demographic and clinical characteristics of the 143 pwCF who underwent serological tests immediately before vaccination, after 3 and 6 months from the 2nd dose of the vaccine as well as the type and severity of adverse reactions. The study population included mainly adults (86%) with a mild to moderate disease as indicated by the percentage of patients with pancreatic insufficiency (55.9%) and the relatively high values of ppFEV1 with most patients having values ≥ 80% predicted. Almost 40% had chronic respiratory infection by P. *aeruginosa*, more than 40% were taking inhaled steroids, around one-third azithromycin or other systemic antibiotics, and one-fourth were being treated with CFTR modulators. Only a minority was taking systemic steroids (n=3, 2.1%). Most patients (95.1%) reported either local and systemic reactions after the 1st or the 2nd dose, which however were of mild severity in half of the patient population and of moderate severity in around 40% of them. None had severe adverse reactions (**Table 1**).

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Table 1. Characteristics of the study population

Number of patients	143 (100)
Male sex	75 (52.4)
Age	
Median (IQR)	21 (18-25)
Adults	123 (86.0)
Age group: 13-17	20 (14.0)
Age group: 18-29	107 (74.8)
Age group: 30-38	16 (11.2)
Pancreatic insufficiency	80 (55.9)
P. aeruginosa infection	57 (39.9)
BMI, kg/m^2, median (IQR)[a]	22.4 (20.1; 24.4)
BMI, z-score, median (IQR) [a, b]	0.10 (-0.55; 0.81))
Underweight[a,c]	8 (5.6)
ppFEV1[d]	
Median (IQR)	97 (82-106)
≥80%	107 (75.9)
40-79%	33 (23.4)
<40%	1 (0.7)
Maintenance therapy	
Inhaled antibiotics	24 (16.8)
Systemic antibiotics	39 (27.3)
Azithromycin	51 (35.7)
Inhaled steroids	65 (45.5)
Systemic steroids	3 (2.1)
CFTR modulators[e]	36 (25.2)
Oxygen therapy	1 (0.7)
SARS-CoV-2 infection	
Prior infection	16
RT-PCR confirmed infection	1
Symptomatic infection	1
Adverse reactions after the 1st or 2nd dose of the BNT162b2 vaccine	
None	7 (4.9)
Local	128 (89.5)
Systemic	103 (72.0)
Mild	71 (49.7)
Moderate	57 (39.9)
Severe	0

Data are expressed as number (%) at least otherwise indicated

[a] BMI was not calculated in one patient due to missing value for height.

[b] For patients aged >20 years, the z-score of BMI was obtained using the reference values corresponding to the age of 20 years.

[c] Underweight was defined according to sex and age-specific z-score of BMI < -1.64 (i.e. < 5th percentile) for patients aged ≤20 years and BMI < 18.5 kg/m^2 for older patients.

[d] Not available for two patients

[e] 31 patients treated with lumacaftor + ivacaftor, 1 patient with tezacaftor + ivacaftor and 4 patients with tezacaftor + ivacaftor + elexacaftor

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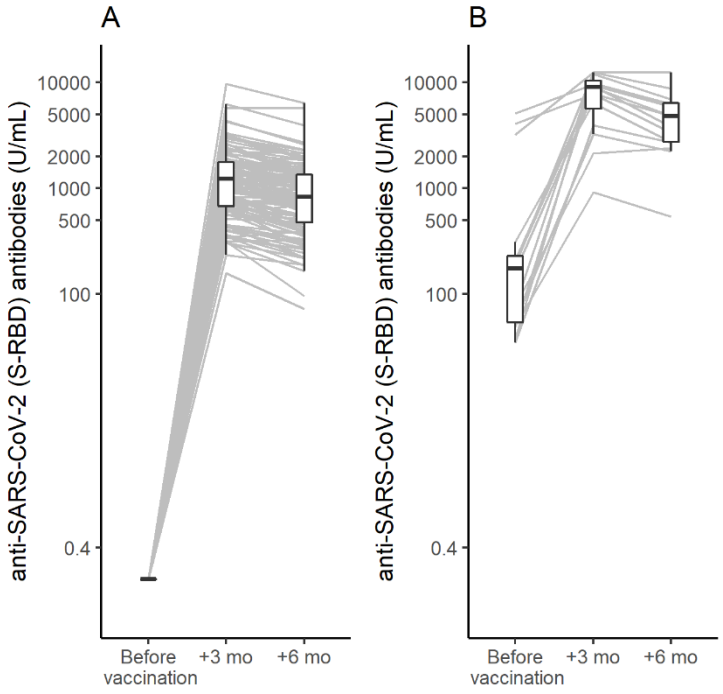
Figure 1 shows the distributions of antibody titers prior to vaccination and after 3 and 6 months from the 2nd injection in patients naïve to SARS-CoV-2 and in those with a previous exposure to the virus. All patients seroconverted (anti-S-RBD≥0.8 U/mL) with antibody titers surging at 3 months from the vaccination in both groups reaching higher values in people with past exposure to SARS-CoV-2 as compared to patients naïve to the virus. At 3 months, median antibody titer (interquartile range, IQR) was 1229 U/mL (677-1772) in patients naïve to the virus and 9107 U/mL (5753-10,418) in those who had been exposed to the virus. At 6 months, antibody titers significantly decreased in both groups with median values of 829 U/mL (475-1342) in patients naïve to the virus and 4810 U/mL (2733-6428) in those previously infected by SARS-CoV-2.



Figures 2-3 show the antibody titer at 3- and 6-month post vaccination according to sex, age groups, exocrine pancreatic status, nutritional status and P. *aeruginosa* infection in people naïve to SARS-CoV-2. There was no difference between sexes, while antibody response decreased with increasing age, although the waning rate was similar across age groups. At 3 months, median values were 1746 U/mL (IQR: 1027-2188), 1265 U/mL (714-1731) and 668 U/mL (432-740) in patients aged <18, 18-29 and ≥ 30 years, respectively. These values decreased at 6 months to 1214 U/mL (834-1710), 812 U/mL (475-1326) and 400 U/mL (279-788), respectively. Patients with pancreatic insufficiency showed a lower antibody response 3-month post-vaccination as compared to those

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with pancreatic sufficiency (854 U/mL, IQR: 571-1513 *vs* 1432 U/mL, IQR: 1220-2096) and a more rapid decline at 6 months (724 U/mL, 354-1237 among patients with pancreatic insufficiency *vs* 1018 U/m, 718-1405 in those with pancreatic sufficiency). Antibodies titers were also lower in patients with P. *aeruginosa* infection (831 U/mL, IQR: 572-1404 at 3 months and 696 U/mL, IQR: 398-1194 at 6 months from vaccination) than in those free from P. *aeruginosa* (1354 U/mL, IQR: 830-1938 at 3 months and 1004 U/mL, IQR: 632-1455 after 6 months from vaccination). The antibody response of underweight patients was comparable to that observed in patients with normal nutritional status.

Figure 2. Antibody titer after 3 and 6 months from the 2nd dose of BNT162b2 mRNA-based vaccine against SARS-CoV-2 in people with cystic fibrosis naïve to SARS-CoV-2 according to sex (Panel A) and age groups (Panel B). Grey symbols indicate individual data, black symbols and error bars show the estimated mean values and corresponding 95% confidence intervals obtained from mixed effect regression models. *P*-values indicate the statistical significance of the main effects of sex and age and their interaction with time (3 or 6 months post vaccination).

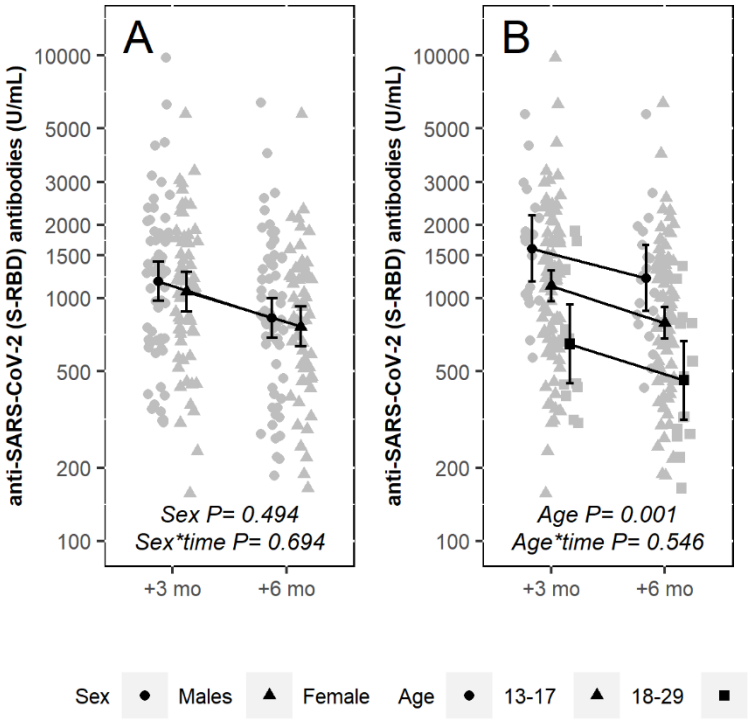

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Figure 3. Antibody titer after 3 and 6 months from the 2nd dose of BNT162b2 mRNA-based vaccine against SARS-CoV-2 in people with cystic fibrosis naïve to SARS-CoV-2 according to exocrine pancreatic status (panel A), underweight (Panel B) and P. *aeruginosa* infection status (Panel C).

Grey symbols indicate individual data, black symbols and error bars show the estimated mean values and corresponding 95% confidence intervals obtained from mixed effect regression models. *P*-values indicate the statistical significance of the main effects of pancreatic insufficiency and P. *aeruginosa* infection and their interaction with time (3 or 6 months post vaccination).

Pa: *Pseudomonas aeruginosa*. PI: Pancreatic insufficiency. S-RBD: Spike Receptor Binding Domain. UNDWT: Underweight.

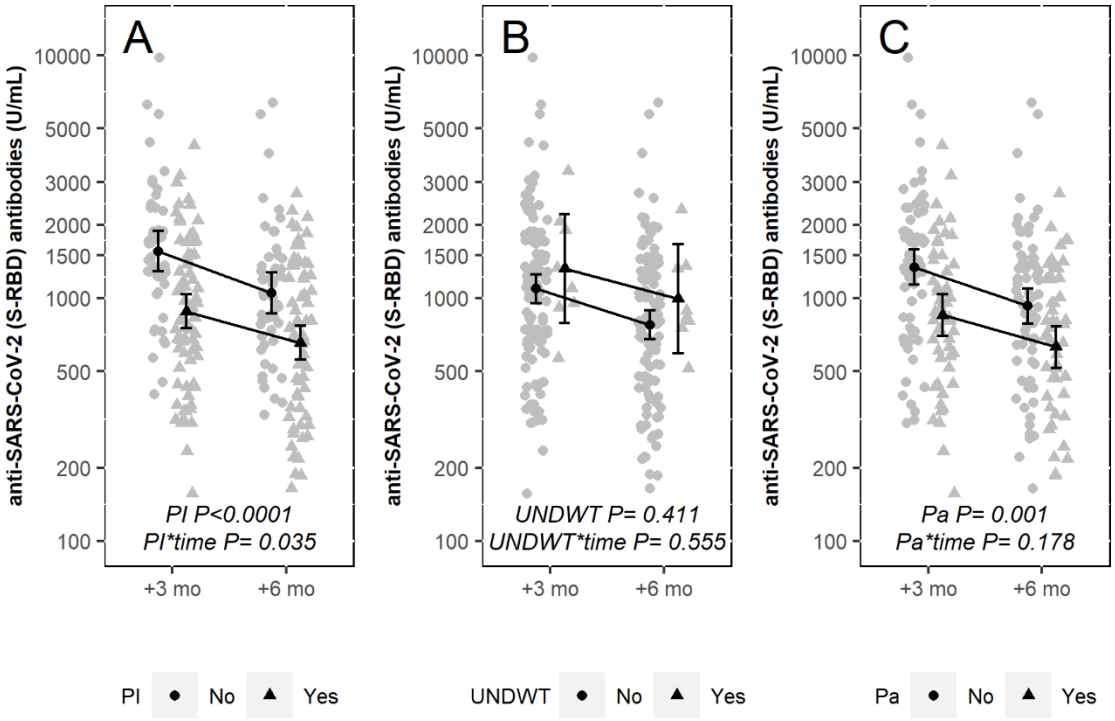

Figure 4-5 depict the antibody response in relation to CF maintenance therapy. There was no significant difference among patients taking inhaled antibiotic or azithromycin and those who did not, while humoral response was lower in patients treated with other systemic antibiotics (943 U/mL, IQR: 638-1369 *vs* 1288 U/mL, IQR: 683-1853 at 3 months and 766 U/mL, IQR: 516-1195 *vs* 855 U/mL, IQR: 475-1361) as well as in those taking inhaled corticosteroids (923 U/mL, IQR: 606-1389 *vs* 1512 U/mL, IQR: 829-2082 at 3 months and 650 U/mL, IQR: 400-1056 *vs* 952 U/mL, IQR: 746-1532 at 6 months) or receiving CFTR modulators (740 U/mL, IQR: 562-1364 *vs* 1293

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U/mL, IQR: 826-1885 at 3 months and 965 U/mL, IQR: 610-1362 *vs* 602 U/mL, IQR: 307-1176 at 6 months). The relationship between oral corticosteroids and antibody response could not be evaluated since only three patients were taking oral corticosteroids and their antibody titers were: 453, 666 and 1883 U/mL after 3 months and 258, 533 and 1473 after 6 months from vaccination.

Figure 4. Antibody titer after 3 and 6 months from the 2nd dose of BNT162b2 mRNA-based vaccine against SARS-CoV-2 in people with cystic fibrosis naïve to SARS-CoV-2 according to cystic fibrosis maintenance therapy: inhaled antibiotics (Panel A), systemic antibiotics (Panel B) and azithromycin (Panel C).

Grey symbols indicate individual data, black symbols and error bars show the estimated mean values and corresponding 95% confidence intervals obtained from mixed effect regression models. *P*-values indicate the statistical significance of the main effects of inhaled antibiotics, systemic antibiotics and azithromycin and their interaction with time (3 or 6 months post vaccination).

AZT: Azithromycin. IAB: Inhaled Antibiotics. SAB: Systemic Antibiotics. S-RBD: Spike Receptor Binding Domain.

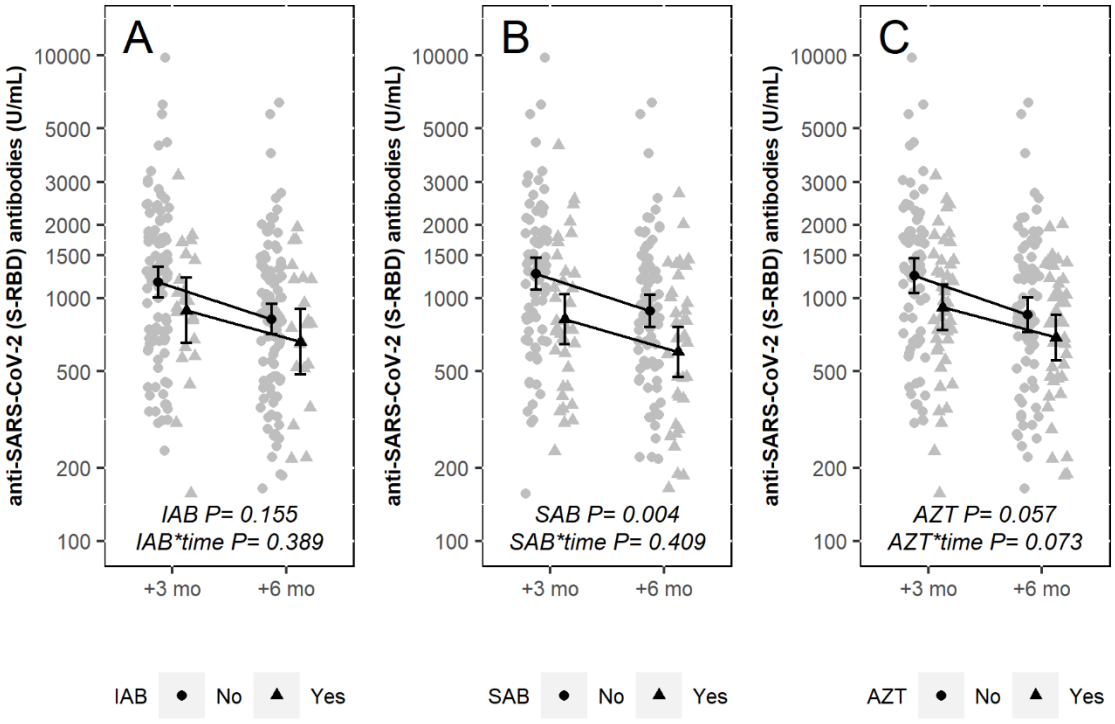

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Figure 5. Antibody titer after 3 and 6 months from the 2nd dose of BNT162b2 mRNA-based vaccine against SARS-CoV-2 in people with cystic fibrosis naïve to SARS-CoV-2 according to cystic fibrosis maintenance therapy: inhaled corticosteroids (Panel A) and CFTR modulators (Panel B).

Grey symbols indicate individual data, black symbols and error bars show the estimated mean values and corresponding 95% confidence intervals obtained from mixed effect regression models. *P*-values indicate the statistical significance of the main effects of inhaled corticosteroids and CFTR modulators and their interaction with time (3 or 6 months post vaccination).

CFTRmod: CFTR Modulators. ICS: Inhaled Corticosteroids. S-RBD: Spike Receptor Binding Domain.

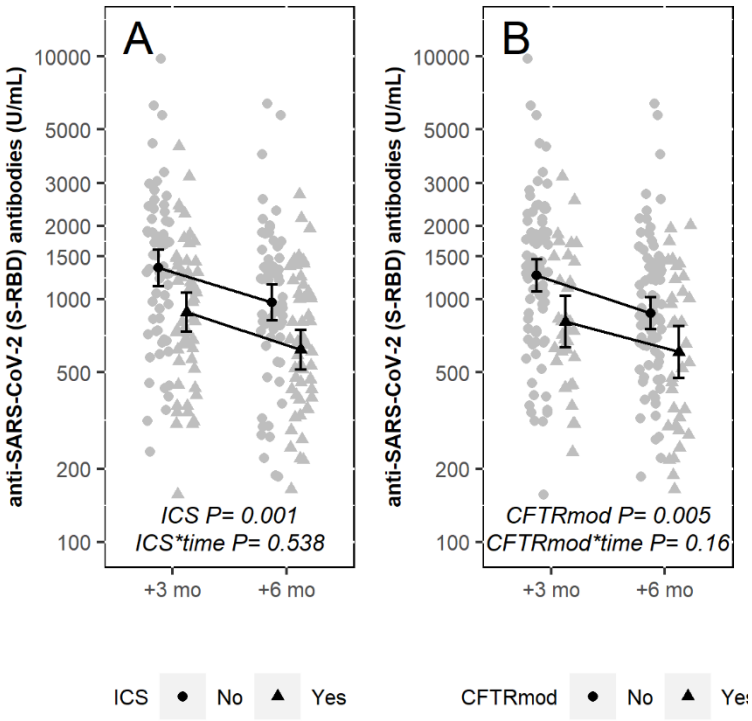

Table 2 gives the results of the model including all the potential determinants of antibody response. Past infection by SARS-CoV-2, age ≥30 years, time of the measurements and treatment with inhaled steroids were the major independent determinants of the antibody response to two doses of BNT162b2 vaccine in pwCF.

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Table 2. Determinants of antibody response to 2nd dose of BNT162b2 mRNA-based vaccine against SARS-CoV-2 in people with cystic fibrosis.

Potential determinant of antibody response	β coefficients[a]	95% CI	*P*-value[b]
Intercept	3.368	(3.231 to 3.505)	
Age group:18-29 vs 13-17	-0.142	(-0.283 to -0.002)	0.047
Age group: 30-38 vs 13-17	-0.354	(-0.552 to -0.157)	<0.001
PI vs PS	-0.089	(-0.215 to 0.037)	0.165
Pa infection (Yes vs No)	-0.081	(-0.200 to 0.037)	0.177
SAB (Yes vs No)	-0.068	(-0.188 to 0.052)	0.267
ICS (Yes vs No)	-0.121	(-0.225 to -0.018)	0.022
CFTRmod (Yes vs No)	-0.046	(-0.169 to 0.078)	0.469
Prior infection by SARS-CoV-2	0.704	(0.552 to 0.857)	<0.001
Time from 2nd injection: 6 vs 3 month	-0.153	(-0.172 to -0.133)	<0.001

CI: Confidence Interval. CFTRmod: CFTR Modulators. ICS: Inhaled Corticosteroids. PI: Pancreatic Insufficiency. Pa: *Pseudomonas aeruginosa*. PS: Pancreatic Sufficiency. SAB: Systemic Antibiotics.

[a] β coefficients indicate the expected difference in mean $\log_{10}$-transformed antibody titer estimated by a mixed effect regression model with subject specific random intercept.

[b] *P*-value indicating whether beta is significant different from 0 (Wald's test).

Type of adverse reactions (systemic vs local/none) and their severity did not significantly affect antibody titers (**Supplementary Figure S1**).

Discussion

In this relatively large population of pwCF who underwent anti-SARS-CoV-2 vaccination, the administration of two doses of the mRNA-based vaccine BNT162b2 induced a strong antibody response, especially among patients previously infected, which however waned within 6 months from the 2nd dose of the vaccine. The antibody titer was highly variable and a few subgroups of the population had lower responses, including patients aged ≥30 years, those with pancreatic insufficiency, P. *aeruginosa* infection and patients regularly treated with systemic antibiotic,

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inhaled corticosteroids or CFTR modulators. When we considered all these factors together, we found that previous infection, age and treatment with inhaled corticosteroids were the only independent predictors of humoral response to two injections of the BNT162b2 vaccine. Thus, indicating that the associations with the remaining factors were largely mediated by age and disease severity.

The antibody titers and their kinetics over 6 months post-vaccination detected in our pwCF were on average comparable to what has been observed in the non-CF populations [12], while the differences observed among age groups in a younger population aged 13-38 years is peculiar to CF . This finding may be at least in part attributed to the progressive nature of CF, characterized by chronic inflammation, frequent respiratory infections, lung damage and prolonged treatments with antibiotics. All these factors may affect the ability of the immune system to mount a humoral response to the vaccine, especially in older patients who are more likely to have a more severe disease.

The higher response detected among patients previously infected by SARS-CoV-2 is comparable to what has been reported in non-CF subjects after the first dose of mRNA-based vaccines that led to the recommendation of a single-dose in pre-exposed healthy individuals [13,14].

The use of corticosteroids over a prolonged period of time is associated with well-known, significant side effects. Patients treated with systemic corticosteroids mount a lower humoral response to vaccination including that against SARS-CoV-2. Studies on patients with musculoskeletal diseases, cancer and on transplant recipients documented that long-term treatment with oral corticosteroids induce a lower antibody response to mRNA-based vaccines [15–17]. However, whether prolonged use of ICS has any immunosuppressive properties and specifically if ICS affects the humoral response to vaccines is unclear, especially in pwCF. Using models of rhinovirus infection, Singanayagam et al. found that the ICS fluticasone propionate impairs innate and acquired antiviral immune responses during virus-induced chronic obstructive pulmonary disease (COPD) exacerbations [18]. However, controversial evidence emerged in studies on

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asthmatic patients where prolonged (≥6 months) treatment with ICS had no effect on cellular immunity [19,20]. Moreover, a study on children and adults with asthma found that use of low dose ICS (≤504 µg/day in adults or 336 µg/day in children of beclomethasone dipropionate equivalent) did not adversely affect the humoral response to influenza A (H1N1, H3N3) vaccine antigens [21]. Similar results were found in a study based on elderly patients with COPD, where patients who received daily treatment with ICS (any dose of beclomethasone, budenoside or fluticasone) showed antibody responses to a MF59-ajuvanted vaccine against influenza strains (A/H1N1, A/H3N and B) comparable to patients who did not receive any steroid treatment [22]. These findings are likely due to the smaller dose adsorbed in patients taking ICS as compared to those treated with oral therapy. However, further investigation is needed to clarify the potential role of ICS in the humoral response to vaccines.

Contrary to what has been documented in a study based on 578 healthcare workers [13] which found a higher antibody response after 12-19 days from BNT162b2 or mRNA-1273 vaccination, we did not observe any relationship between the occurrence of systemic reactions and antibody titer. While, the lack of association between symptoms severity and vaccine-induced antibody response was previously reported in a study based on 206 healthy adults with no history of Covid-19 [23].

A potential link between malnutrition and vaccine responsiveness in pwCF has been suggested by Launay et al. who found a lower immune response to vaccine against 2009 pandemic A/H1N1 in pwCF with low BMI [3]. We could not confirm this finding, however there were only 8 underweight patients in our cohort.

When interpreting our results, it should be noted that the decreased antibody titers after 6 months from vaccination does not necessarily reflect a reduced protection against infection and severe Covid-19. In fact, T cell response, antiviral B and T cell memory, not measured in our study, have proven to be important in the maintenance of SARS-CoV-2 immunity despite the drop in circulating antibodies [24–27].

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Our study provides unique data on the durability and dynamics of humoral responses to the most frequently administered mRNA-based vaccine among the CF population. Our results are of clinical relevance since the levels of antibody binding the S-RBD antigen measured post-vaccination are related to a lower probability of infection [28].

However, our population of pwCF is characterized by mild to moderate disease and we could not evaluate the antibody response in patients with severe CF, including those with end-stage lung disease and transplanted patients, with the latter expected to have a lower humoral response due to the strong immunosuppressive therapies. Another limitation of our study is the short-term evaluation of the durability of antibody response (6 months). Finally, our data were collected when two doses of the vaccine had been administered, but at the time of writing all patients have received the third dose, the immunogenicity of which in this population remains to be determined.

Conclusions

Immunogenicity of BNT162b2 was comparable to that observed in the general population. However, we have found a marked heterogeneity in our patients, with lower humoral responses in patients aged ≥30 years and those using ICS. Future studies focusing on mechanisms other than antibody production are needed to understand the durability of immunization in pwCF also in view of the new emerging variants that seem to partly escape from the antibody neutralization [29–31].

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of the IRCCS, Istituto Nazionale per le Malattie Infettive, Lazzaro Spallanzani, Rome, Italy (protocol number: 354 2020/2021).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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Data availability statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy and ethical restrictions.

Conflicts of interest statement: The authors declare no conflict of interest.

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Author contributions:

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