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Immune signature in vaccinated versus non-vaccinated aged people with COVID-19 pneumonia

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Abstract

Background A definition of the immunological features of COVID-19 pneumonia is needed to support clinical management of aged patients. In this study, we characterized the humoral and cellular immune responses in presence or absence of SARS-CoV-2 vaccination, in aged patients admitted to the IRCCS San Raffaele Hospital (Italy) for COVID-19 pneumonia between November 2021 and March 2022.

Methods The study was approved by local authorities. Disease severity was evaluated according to WHO guidelines. We tested: (A) anti-SARS-CoV-2 humoral response (anti-RBD-S IgG, anti-S IgM, anti-N IgG, neutralizing activity against Delta, BA1, BA4/5 variants); (B) Lymphocyte B, CD4 and CD8 T-cell phenotype; (C) plasma cytokines. The impact of vaccine administration and different variants on the immunological responses was evaluated using standard linear regression models and Tobit models for censored outcomes adjusted for age, vaccine doses and gender.

Result We studied 47 aged patients (median age 78.41), 22 (47%) female, 33 (70%) older than 70 years (elderly). At hospital admission, 36% were unvaccinated (VAC_{no}), whilst 63% had received 2 (VAC_2) or 3 doses (VAC_3) of vaccine. During hospitalization, WHO score > 5 was higher in unvaccinated (14% in VAC_3 vs. 43% in VAC_2 and 44% VACno). Independently from vaccination doses and gender, elderly had overall reduced anti-SARS-CoV-2 humoral response (IgG-RBD-S, $p = 0.0075$). By linear regression, the anti-RBD-S ($p = 0.0060$), B ($p = 0.0079$), CD8 ($p = 0.0043$) and Th2 cell counts ($p = 0.0131$) were higher in VAC_{2+3} compared to VAC_{no}. Delta variant was the most representative in VAC_2 ($n = 13/18$, 72%), detected in 41% of VAC_{no}, whereas undetected in VAC_3, and anti-RBD-S production was higher in VAC_2 vs. VAC_{no} ($p = 0.0001$), alongside neutralization against Delta ($p = 0141$), BA1 ($p = 0.0255$), BA4/5 ($p = 0.0162$). Infections with Delta also drove an increase of pro-inflammatory cytokines (IFN-α, $p = 0.0463$; IL-6, $p = 0.0010$).

Conclusions Administration of 3 vaccination doses reduces the severe symptomatology in aged and elderly. Vaccination showed a strong association with anti-SARS-CoV-2 humoral response and an expansion of Th2 T-cells

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populations, independently of age. Delta variants and number of vaccine doses affected the magnitude of the humoral response against the original SARS-CoV-2 and emerging variants. A systematic surveillance of the emerging variants is paramount to define future vaccination strategies.

Keywords Elderly, COVID-19 vaccine, Non-vaccinated, SARS-CoV-2 variants, Immunological response, Plasma cytokines, COVID-19 disease severity, Th2, Pneumonia

Introduction

SARS-CoV-2 infection can lead to COVID-19 pneumonia. The risk of death in the general population is low but it dramatically increases in elder individuals with comorbid chronic conditions such as hypertension, cardiovascular diseases, type 2 diabetes, and with obesity, smoking habits, and male gender [1, 2]. Furthermore the immune system undergoes remarkable changes known as immunosenescence during aging. A low-grade chronic inflammation, known as "inflamm-aging", causes a progressive decline in the ability to produce effective humoral and cellular responses against infections or upon vaccination [3, 4]. In aged individuals, a hyper-inflammatory condition is favoured by the chronic activation of monocytes, which generates a pro-thrombotic environment, contributing to the negative outcomes observed in severe COVID-19 [5, 6]. Moreover, the alteration of T lymphocytes repertoire with aging [6] can affect the accessibility of naïve T cells to SARS-CoV-2 antigens, reducing the activation of specific cells [7]. Also, long-lived B cell repertoire, important in maintaining immunity elicited by vaccines [8–10] is affected by immunosenescence and a particular sign of this impairment is the expansion of the atypical non-functional B cells that is associated with suboptimal humoral responses to vaccine [11].

Despite ongoing immunosenescence in elderly population, the administration of COVID-19 vaccine has demonstrated efficacy [8, 12–15] with an incredible impact on the prevention of severe disease [16–19]. A milder course of the disease is a reflection of a prompt anti-SARS-CoV-2 immune response elicited by the vaccine, which decreases the progression of the infection and supports a quicker virus clearance, preventing the raise of COVID-19 complications. There are scarce data reporting comprehensive immunological characterization in elderly patients. One previous study described the immune features in 31 aged patients with severe SARS-CoV-2 infection (mean age: 76.4 years), compared to 33 adult patients at the same stage of infection (mean age: 49.8 years) in absence of vaccination [6]. Whilst success of vaccination is not questioned, it still remains to better characterize the immunological mechanisms associated with severe COVID-19, even after SARS-CoV-2 vaccination.

In this study, we provided a fine characterization of the humoral and cellular immune responses in aged patients who were admitted to the IRCCS San Raffaele Hospital (Italy) for COVID-19 pneumonia, between November 2021 and March 2022, during Delta/Omicron variants of concern (VOC) waves. We compared unvaccinated patients with subjects receiving two or three doses, to evaluate the impact of vaccination on the immunological humoral, cellular and pro-inflammatory response. This population is unique, having a group of patients that were naïve to vaccination or infection.

Methods

Study population

This study included a total of 47 patients (median age 78.41, ranging from 60 to 94 years old) admitted to the IRCCS San Raffaele Hospital (Milan, Italy) for pneumonia between November 2021 and March 2022. All patients had proven evidence of SARS-CoV-2 infection with nasopharyngeal swab tested positive for SARS-CoV-2 nucleic acid using reverse-transcriptase real-time PCR assay [20, 21], and they were treated with corticosteroids according to common clinical practice. Disease severity was evaluated according to WHO guidelines [22]: score (s) ≤ 5 = moderate; s > 5 = severe. The study group included vaccinated and non-vaccinated patients. Information about vaccine type is available only for 18/30 (60%). Patients receiving 2 doses were vaccinated with Pfizer Comirnaty vaccine (8/16, 50%), or with Astra Zeneca Vaxzevria (5/16, 31%), or with Moderna Spikevax (3/16, 19%). Information about the booster dose were available only for two patients (one received two doses of Astra Zeneca Vaxzevria and one Pfizer Comirnaty dose; the other received 3 Pfizer Comirnaty doses). Individuals that needed intensive care unit (ICU) support at admission were excluded. Two patients were admitted with score = 6 and included in the study, but they experienced a severe curse the infection and died during hospitalization.

SARS-COV-2 VOC genomic characterization

SARS-COV-2 sequences were obtained using Menarini Diagnostics CoronaMeltVAR Real Time PCR kit (Firenze, Italia). Viral genome characterization of the SARS-CoV-2 VOC driving the pneumonia was available for only 17 subjects. For the other patients, we estimated the probable VOC based on the genomic epidemiological data of the Italian National Institute of Health [23]. According to Lombardy epidemiological data, Omicron surpassed Delta and became the most prevalent VOC at the beginning of January 2022 [24]. Considering a reasonable

time-lag between infection and hospitalization of about 7–10 days [25], patients hospitalized after the 15 of January 2022 were considered as Omicron-infected.

Sample collection and storage

EDTA-venous blood and serum were collected within 3 days after hospital admission, with all patients having received corticosteroids. Plasma was isolated from EDTA-blood and stored at -80^{0}C for further use. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll density gradient and cryopreserved in FBS 10% DMSO until analysis, in liquid nitrogen. Serum was aliquoted and stored at -80^{0}C until use.

Anti-SARS-CoV-2 humoral response

All individuals were tested for IgG recognizing the RBD domain of the Spike glycoprotein (IgG-RBD-S), IgG against the Nucleocapsid protein (IgG-N) and IgM against the Spike glycoprotein (IgM-S). IgM-S and IgG-N were measured using the SARSCoV-2 IgG-N and the SARS-CoV-2 IgM-S assays (Abbott, Ireland), respectively, and IgG-RBD-S were tested using the SARS-CoV-2 IgG II Quant assay (Abbott, Ireland). Samples were run in single replicate according to the manufacturer's instructions, using the ARCHITECT i2000 System (Abbott), as previously described [26–28]. For IgG-N and IgM-S, the results were reported as assay index (S/C) with a positive cut-off ≥1.4 for IgG-N and ≥1 for IgM-S. For IgG-RBD-S results were reported as binding antibody Unit/mL (BAU/mL, cut-off ≥7.1) [29]. Samples with values>5680 BAU/mL (upper limit of quantification) were diluted 1:2 and measured again. Concentrations were reported considering the dilution factor.

SARS-CoV-2 neutralizing activity against virus variants Delta, BA1, BA4/5

Neutralizing activity of sera was tested using lentiviral pseudotypes of SARS-CoV-2, as previously described [30–32]. SARS-CoV-2 pseudotypes with Spike variants were produced in HEK293T/17 cells (human embryonic kidney 293 cells, ATCC CRL-11268) by co-transfecting with the Spike variant-coding plasmids, packaging plasmid p8.91 and pCSFLW reporter plasmid using the FuGENE HD Transfection Reagent (Promega) according to the manufacturer's instructions. Supernatants containing the virus were harvested 72 h after transfection, centrifuged at 500xg for 5 min to clear it from cell debris and filtered with a 0.45-µm filter before storage at −80 °C. Before neutralization, all virus stocks were titrated by infection of Chinese Hamster Ovary (CHO) cells that stably expressed human ACE2/TMPRSS2 proteins (CHO ACE2/TMPRSS2, herein referred to as CHO/A2/T2) which are the cellular targets of SARS-CoV-2 infection, as described previously [30, 33]. Sera neutralizing potency was assayed on CHO/A2/T2 cells. Endpoint two-fold serial dilutions of heat-inactivated sera samples (56 °C for 30 min) were incubated with 106 RLU of pseudotyped viruses at 37 °C 5%, CO2 for 1 h before addition of 104 CHO/A2/T2 cells per well (96 well plate format) and incubation for 48 h. Following incubation, cells were lysed in Luciferase Assay System (Promega) and luciferase activity was measured using a Glo-Max luminometer (Promega). The neutralization rates were expressed as IC50 values, defined as the inhibitory dilution at which the half-maximal neutralization is achieved. To set up the neutralization assay the International Standard for anti-SARS-CoV-2 antibody (NIBSC code 20/136) and WHO Reference Panel were included as controls, as established previously [30].

Immune cell phenotype (B, CD4 and CD8)

Cellular markers were measured by staining frozen PBMCs. For B and T cell populations DURAClone IM B cell tube and DURAClone IM T cell (both from Beckman Coulter, Research Use Only RUO) were used as we previously described [34]. Using surface marker staining, we assessed the frequencies of B and T cell maturation stage distribution. Therefore, we examined the exhausted or senescent phenotype of T cells, by measuring respectively the Programmed cell death protein 1 (PD-1) and CD57 expression on the cells' surface. Moreover, we designed two panels for helper (Th) and regulatory (Treg) T cells, as previously published [27]. Data acquisition was performed using a CytoFlex flow cytometer with CytExpert v2.3 software (Beckman Coulter). The stopping rule was set at 10,000 events in the T cells (CD3+) panel and 1,000 events in the B-cells (CD19+). Data were analysed with Kaluza v2.1 software (Beckman Coulter) and the Cytobank Premim software (Beckman Coulter). The list of Ab and gating strategies applied were described in Supplementary Tables 1 and Supplementary Figs. 1, 2, 3.

Plasma cytokine profile

MACSPlex Cytokine 12 kit human (MACS Miltenyi Biotec) was used as indicated by the manufacturer to specifically detect: GM-CSF, IFN- α, IFN-γ, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p70, IL-17α e TNF- α. Data were acquired on a CytoFlex flow cytometer (Beckman Coulter) at a flow rate of 20 µL/minute. The acquisition stopping rule was set to 4.000 events in the bead gate or 180 µl of acquired sample. The exported data were analyzed with Flowlogic software (Inivai Technologies). Cytokines' concentration (pg/ml) was obtained by interpolation with the standard curve provided by the kit.

Statistical analysis

Results for continuous variables were summarized using median and IQR while categorical variables using

frequencies and percentages. Nonparametric tests were applied to compare patients receiving different vaccine doses for relevant demographic/clinical characteristics and immunological responses: in particular, Fisher's exact test was used with categorical variables, while the Mann-Whitney test was applied in continuous variables. Spearman's partial correlation coefficient was calculated to evaluate, within patients receiving 0 or 2/3 doses of vaccine, the presence of a monotonic relationship between two immunological responses after adjusting for age. The false discovery rate (FDR) approach was used to adjust *p*-values thus addressing arising multiplicity issues. Multiple regression models were performed to evaluate differences among groups defined either (i) on received dose or (ii) on age (>70 yrs vs. ≤ 70 yrs) on immunological response adjusting for potential confounding variables. In particular, along with standard linear regression models, Tobit models have been estimated in the presence of censored dependent outcome variables. To satisfy underlying model assumptions, outcome variables were transformed using standard transformations (e.g., logarithm, power transformation, square root, ordered quantile normalization). All the analyses were performed using R statistical software (version 4.2.2, https://cran.r-project.org/index.html). In all the analyses, the significance level was set at 0.05.

Results

Patients' population characteristics according to vaccination doses and age

The study cohort included 47 patients hospitalized for COVID-19 pneumonia resulting from SARS-CoV-2 infection, during Delta and Omicron waves. Patients' characteristics are shown in Table 1. Overall, median age was 78.41 years [IQR 68–84], 22/47 (47%) were female, and 12/47 (29%) had history of cancer. Based on the WHO clinical progression scale [22], 25/47 (57%) patients were classified as moderate (score 4 and 5, s≤5) and 19/47 (43%) as severe patients (score 6, s>5). Patients that appeared critically ill at admission and needed ICU were not included in the study. Apart from 2 patients (age>80 years), who experienced a negative progression of the disease and died (at admission s>5; at death s=10), all the other patients achieved a full remission.

At hospital admission, 17/47 (36%) individuals were not vaccinated (VAC_{no}), whilst the remaining 30/47 (64%) had received 2 doses (18/30, 60%, VAC_2) or 3 doses (12/30, 40%, VAC_3) of anti-SARS-CoV-2 vaccine, designed *versus (vs.)* the original Wuhan strain. Comparing general characteristics of vaccinated and unvaccinated patients, the ratio male/female was similar in the two groups (VAC_{no}vs. VAC_{2+3}), while the VAC_{2+3} one was relatively older than the VAC_{no} (medians years 80 vs. 71, respectively). The older group (VAC_{2+3}) was more likely to have experienced some comorbidities compared to the younger one (VAC_{no}), including obesity (19% vs. 0%), chronic obstructive pulmonary disease (COPD, 4% vs. 0), diabetes (12% vs. 6%), cancer (31% vs. 25%), or other diseases (31% vs. 13%, specified in Table 1), albeit none of the difference was statistically significant. Both VAC_{2+3} and VAC_{no} experienced pulmonary arterial hypertension (PAH) with similar frequency (39% vs. 38%). Within VAC_{2+3} group, individuals who received 3 vaccination doses were less likely to have experienced PAH (10% of cases in VAC_3 vs. 56% in VAC_2). With regards to the percentage of severe patients (s>5), this was lower in VAC_{2+3} group (39%) than in VAC_{no} (50%) and among the vaccinated, those with three doses were less likely to have experienced severe symptoms (30% of s>5 in VAC_3 vs. 44% in VAC_2) (Table 1).

Anti-SARS-CoV-2 response in elderly versus aged patients

Further, we explored the impact of age on the disease outcome and immune response in the context of COVID-19 pneumonia in presence or absence of vaccination. We thus divided the population into two strata: one below 70 years of age (≤70y, $n=14$) and one over 70 (>70y, $n=33$) and the characteristics of these 2 groups are provided in Supplementary Table 2. As it could be expected, the individuals>70y were more likely to have experienced comorbidities associated with aging such as PAH (29% in ≤70y vs. 36% in >70y), diabetes (absent in ≤70 vs. 12% in >70y), cancer (21% in ≤70y vs. 27% in >70y) and other diseases (14% in ≤70 vs. 24% in >70y). Lack of vaccination was more frequent in younger individuals, with 50% of ≤70y and 30% of >70y subjects being VAC_{no}. Of note, in the elderly group, administration of three doses of vaccination resulted in a lower proportion of severe cases (14% severe cases in VAC_3 vs. 43% in VAC_2 and 44% in VAC_{no}). We further run a multivariable regression model comparing subjects≤70 yrs and >70 yrs of age adjusted for vaccine doses and gender (Table 2). We found that elderly had an overall lower anti-SARS-CoV-2 humoral response (IgG-RBD-S) with an expansion of CD28null CD4 populations. Of note, none of the individuals who received 3 doses experienced death, whilst the two people who died were both >70y: one was VAC_2 and the other was VAC_{no} (Supplementary Table 2).

Vaccination was associated with increased anti-SARS-CoV-2 humoral response and neutralizing activity

Humoral response was evaluated by measuring circulating IgG-N, IgM-S, IgG-RBD-S Antibodies (Ab) (Table 3; Fig. 1). Overall, IgG-N were detectable in 33/47 (70%), IgM-S in 26/47 (55%) and IgG-RBD-S in 30/47 (81%). By linear regression models adjusted for gender, age and cancer, comparing individuals that received or not the vaccine, we reported that IgG-RBD-S Ab levels

Table 1 Patients' characteristics

		All	VAC_{no}	VAC_{2+3}		VAC_2		VAC_3	
		47	*17*	*30*	p-value (2 + 3 vs. no)	*18*	p-value (2 vs. no)	*12*	p-value (3 vs. 0)
WHO classification, n (%)	**Moderate, s ≤ 5**	25 (56.8)	8 (50.0)	17 (60.7)	0.54	10 (55.6)	1	7 (70.0)	0.428
	Severe, s > 5	19 (43.2)	8 (50.0)	11 (39.3)		8 (44.4)		3 (30.0)	
	NA, n	3	1	2				2	
PAH, n (%)	**No**	26 (61.9)	10 (62.5)	16 (61.5)	1	7 (43.8)	0.479	9 (90.0)	0.19
	Yes	16 (38.1)	6 (37.5)	10 (38.5)		9 (56.2)		1 (10.0)	
	NA, n	5	1	4		2		2	
Obesity, n (%)	**No**	37 (88.1)	16 (100.0)	21 (80.8)	0.138	11 (68.8)	0.043	10 (100.0)	1
	Yes	5 (11.9)	0 (0.0)	5 (19.2)		5 (31.2)		0	
	NA, n	5	1	4		2		2	
COPD, n (%)	**No**	41 (97.6)	16 (100.0)	25 (96.2)	1	16 (100.0)	1	9 (90.0)	0.385
	Yes	1 (2.4)	0 (0.0)	1 (3.8)		0		1 (10.0)	
	NA, n	5	1	4		2		2	
Diabetes, n (%)	**No**	38 (90.5)	15 (93.8)	23 (88.5)	1	13 (81.2)	0.6	10 (100.0)	1
	Yes	4 (9.5)	1 (6.2)	3 (11.5)		3 (18.8)		0	
	NA, n	5	1	4		2		2	
Cancer, n (%)[a]	**No**	30 (71.4)	12 (75.0)	18 (69.2)	0.74	13 (81.2)	1	5 (50.0)	0.234
	Yes	12 (28.6)	4 (25.0)	8 (30.8)		3 (18.8)		5 (50.0)	
	NA, n	5	1	4		2		2	
Other diseases, n (%)[b]	**No**	32 (76.2)	14 (87.5)	18 (69.2)	0.27	12 (75.0)	0.654	6 (60.0)	0.163
	Yes	10 (23.8)	2 (12.5)	8 (30.8)		4 (25.0)		4 (40.0)	
	NA, n	5	1	4		2		2	
Gender, n (%)	**F**	22 (46.8)	9 (52.9)	13 (43.3)	0.558	7 (38.9)	0.505	6 (50.0)	1
	M	25 (53.2)	8 (47.1)	17 (56.7)		11 (61.1)		6 (50.0)	
Outcome, n (%)	**RE**	45 (95.7)	16 (94.1)	29 (96.7)	1	17 (94.4)	1	12 (100.0)	1
	DE	2 (4.3)	1 (5.9)	1 (3.3)		1 (5.6)		0 (0.0)	
SARS-CoV-2 VOC, n (%)	**Delta**	20 (42.6)	7 (41.2)	13 (43.3)	1	13 (72.2)	0.092	0 (0.0)	0.023
	Omicron	27 (57.4)	10 (58.8)	17 (56.7)		5 (27.8)		12 (100.0)	
age (median [IQR])		78.41 [68.31, 84.04]	71.32 [66.78, 79.51]	79.80 [74.15, 84.27]	0.163	79.24 [74.15, 82.28]	0.276	80.78 [74.58, 84.37]	0.184

NA = not available data; RE: remission, DE: death. PAH: pulmonary arterial hypertension. COPD: chronic obstructive pulmonary disease. [a]Type of cancer in the population were: chronic lymphatic leukaemia ($n=2$), lymphoma ($n=1$), multiple myeloma ($n=1$), myelofibrosis ($n=1$), breast cancer ($n=2$), pancreatic cancer ($n=1$), lung cancer ($n=1$), colorectal cancer ($n=1$), prostatic cancer ($n=1$), adenoid cystic carcinoma ($n=1$). [b]other disease included: cardiovascular ($n=2$), pulmonary ($n=2$), metabolic (other than diabetes and obesity, $n=1$), renal ($n=1$), neurologic ($n=4$). p-values referred to Fisher's exact test in presence of categorical outcomes, while Mann-Whitney test was applied in the presence of continuous variables

were higher in VAC_{2+3} compared to VAC_{no} ($p=0.0026$, Table 3; Fig. 1) conversely to what was observed for IgG-N Ab levels which were lower in (VAC_{2+3} compared to VAC_{no} ($p=0.0408$, Table 3; Fig. 1. IgM-S levels did not vary across the groups.

In a separate regression model, using the same adjustments described above, we evaluated the impact of one or two doses of vaccine, and we compared the humoral response in VAC_{no} vs. VAC_2 or VAC_3 (Supplementary Table 3). We observed that both VAC_2 and VAC_3 had higher levels of IgG-RBD-S compared to VAC_{no}, but this was only significant for VAC_2 ($p=0.0001$). On the other hand, anti-N IgG levels decrease with the number of vaccine doses, with the highest level detected in VAC_{no} group ($p=0.0014$ compared with VAC_3), as showed in Fig. 1; Table 3.

Further, we explored the impact of vaccination on the Ab neutralization activity during natural infection driving pneumonia. We tested neutralizing antibodies against both circulating variants Delta, BA.1 and BA.4/5 and human seasonal coronaviruses (HCOVs, 229E, HKU1, NL63). Overall, individuals who received vaccination (VAC_{2+3}) showed significantly higher levels of neutralizing activity against the circulating variants compared to VAC_{no} ($p=0.0.34$ Delta; $p=0.044$ BA.1 and $p=0.038$ BA.4/5; Table 3). Of note, this difference was mainly driven by VAC_2, rather than VAC_3 (Supplementary Table 3). Activity versus seasonal coronaviruses was not different between the groups.

Cellular immune response was elevated in individuals who received vaccination, regardless to age, gender or cancer history.

Table 2 Multiple regressions for comparison of subjects > 70 yrs of age and subjects ≤ 70 yrs of age, adjusted for vaccine doses and gender. Tobit regression models for IgG-N (index), IgM-S (index) and IgG-RBD-S (BAU/mL). Linear regression models for the other outcomes

	> 70 yrs vs. ≤ 70 yrs		
Outcome	**Estimate**	**Std.er.**	***p*-value**
IgG-N (index)	-2.0165	0.7431	0.0067
IgM-S (index)	-1.2085	0.7847	0.1236
IgG-RBD-S (BAU/mL)	-2.2159	0.8287	0.0075
IC50 229E	0.2193	0.3314	0.5119
IC50 HKU1	0.1482	0.3278	0.6535
IC50 NL63	-0.1728	0.3452	0.6193
IC50 DELTA	-0.7378	0.2877	0.0141
IC50 BA1	-0.5189	0.3042	0.0956
IC50 BA4/5	-0.5859	0.2954	0.0541
GM-CSF pg/ml	-0.2694	0.4097	0.5148
IFN-α pg/ml	-0.1027	0.3306	0.7577
IFN-γ pg/ml	0.0584	0.3191	0.8558
IL-4 pg/ml	-4.3309	13.8644	0.7565
IL-5 pg/ml	-0.1588	0.3146	0.6168
IL-6 pg/ml	0.5311	0.3135	0.0985
IL-10 pg/ml	0.2305	0.7504	0.7604
IL-12p70 pg/ml	-0.1541	0.4480	0.7327
IL-17 A pg/ml	-0.1109	0.4508	0.8070
TNF-α pg/ml	-0.0881	0.3039	0.7733
leukocytes	-0.3116	0.3455	0.3727
B cells count	-0.2466	0.3113	0.4330
B cells (% on CD45+)	-0.5055	0.6985	0.4736
B activated (CD19+/CD27+/IgD-/CD21-)	0.6580	0.3130	0.0440
B resting (CD19+/CD27+/IgD-/CD21+)	0.7319	0.5651	0.2051
CD21low/CD38 low (CD19+/ $CD21^{low}$/$CD38^{low}$)	-0.3782	0.3683	0.3127
DN (CD19+/CD27-/IgD-)	0.4236	0.4014	0.2998
Marginal Zone (MZ) (CD19+/CD27+/IgD+)	0.0240	0.2882	0.9341
Memory B cells (MB) (CD19+/CD27+/IgD-)	0.9430	0.5817	0.1154
Naive B cells (CD19+/CD27-/IgD+/CD21-)	-13.0766	8.8247	0.1488
Plasmablast (CD19+/CD27+/IgM-/IgG-/$CD38^{high}$)	7.6308	4.9079	0.1305
SWI (CD19+/CD27+/IgM-/IgG-)	0.8061	0.6244	0.2065
TLM (CD19+/CD27-/CD21-)	-0.3157	0.4136	0.4512
Trans B (CD19+/CD27-/ $CD38^{high}$/$CD24^{high}$)	-0.5682	0.3394	0.1045
UNSWI (CD19+/CD27+/IgM+/IgG+)	0.0692	0.5991	0.9089
CD4 + T cells count	0.0321	0.3353	0.9242
CD4+ (% of CD3+)	-4.9385	6.4672	0.4497
CM-CD4 (CD4+/CD45RA-/CCR7+)	-3.5265	4.4790	0.4364
N-CD4 (CD4+/ CD45RA+ /CCR7+)	-2.6626	4.1079	0.5211
EM-CD4 (CD4+/ CD45RA-/ CCR7-)	4.5056	4.3223	0.3044
TEMRA CD4 (CD4+/ CD45RA+/ CCR7+)	0.6308	0.5071	0.2218
CD4+/PD1-/CD57-	-5.6604	5.7776	0.3340
CD4+/PD1-/CD57+	0.3004	0.3344	0.3750
CD4+/PD1+/CD57-	0.1981	5.1764	0.9697
CD4+/PD1+/CD57+	0.7492	0.4763	0.1247
CD4+/CD27-/CD28-	0.7354	0.3063	0.0218
CD4+/CD27-/CD28+	0.2070	0.2981	0.4921
CD4+/CD27+/CD28-	0.7004	0.3400	0.0469
CD4+/CD27+/CD28+	-8.2945	5.1942	0.1193
CD8 + T cells count	1291.8860	632.4504	0.0479
CD8+ (%of CD3+)	0.0853	0.1746	0.6280

Table 2 (continued)

	> 70 yrs vs. ≤ 70 yrs		
Outcome	**Estimate**	**Std.er.**	***p*-value**
N-CD8 (CD8+/ CD45RA+ /CCR7+)	-0.3726	0.2208	0.1001
CM-CD8 (CD8+/CD45RA-/CCR7+)	-8.0210	6.3046	0.2114
EM-CD8 (CD8+/ CD45RA-/ CCR7-)	7.8387	5.3385	0.1507
TEMRA-CD8 (CD8+/ CD45RA+/ CCR7+)	0.9748	0.6742	0.1568
CD8+/CD57-/PD1-	0.2013	2.9723	0.9464
CD8+/CD57-/PD1+	-0.1358	0.1691	0.4273
CD8+/CD57+/PD1-	0.6822	0.5927	0.2574
CD8+/CD57+/PD1+	1.7821	5.0780	0.7277
CD8+/CD27-/CD28-	4.4434	6.7942	0.5173
CD8+/CD27-/CD28+	0.4703	0.2475	0.0655
CD8+/CD27+/CD28-	1.4167	1.7725	0.4294
CD8+/CD27+/CD28+	-8.8544	6.4304	0.1770
Th1 (CD4+/CCR6- /CXCR3+);	4.1130	4.3099	0.3463
Th17 (CD4+/CCR6+/ CCR4+)	0.1043	0.3537	0.7698
Th17-1 (CD4+/CCR6+/CXCR3+)	-0.4384	0.4224	0.3062
Th2 (CD4+/CCR6-/CCR4+)	-0.2955	0.3311	0.3781
Treg (CD4+/CD25+/CD127^{low})	0.1662	0.1639	0.3175

At admission, extensive phenotypic profiling was also performed to evaluate the immune activation in the B and T (CD4, CD8) cell compartments. All the cellular subpopulations were included in the linear regression models and reported in Table 3 and Supplementary Table 3.

With regards to B-cells, we observed only that the total B count was higher in VAC_{2+3} compared to VAC_{no} ($p=0.0079$), meanwhile none of the activated populations were different (Table 3 and Supplementary Table 3). This data was probably driven mostly by the comparison VAC_2 vs. VAC_0 (Supplementary Table 3, $p=0.0028$). When looking at the CD4 sub-populations in the three vaccination groups, levels of Th1 lymphocytes (CCR6-/CXCR3+) appeared to be the most abundant compared to the other Th subtypes (Th2, Th17-1, Th17) (Fig. 2A-B, Supplementary Table 3). Of note, the proportion of the Th2 cell varied across the groups, with the VAC_{2+3} showing higher levels compared to VAC_{no} ($p=0.009$, Fig. 2A; adjusted value in Table 3). This difference remained significant also when the number of vaccine doses was considered. Indeed, both VAC_2 ($p=0.0233$) and VAC_3 ($p=0.0241$) had higher levels compared to VAC_{no} (adjusted values in Supplementary Table 3, Fig. 2B). We did not observe significant differences regarding the other CD4 populations, a part of an increase of the effector memory CD4 in the VAC_3 compared to VAC_{no} (EM-CD4+, $p=0.0325$, Supplementary Table 3).

Finally, we explored the CD8 population and we found an increase of the proportion of CD8 in VAC_{2+3} compared to VAC_{no} ($p=0.008$, Table 3) and this association persisted only when comparing separately VAC_{no} to VAC_3 ($p=0.0319$, Supplementary Table 3); furthermore, individuals who received 3 vaccine doses also had higher total CD8 counts ($p=0.0002$) compared to unvaccinated (Supplementary Table 3). When looking at the CD4/CD8 lymphocytes ratio (Fig. 2C), consistently with the multivariable adjusted analysis, we observed an expansion of the CD8 in VAC_3 group.

Soluble cytokines levels during COVID-19 pneumonia varied according to vaccination doses

Alongside the characterization of humoral and cellular responses of our cohort, we also profiled the serum levels of cytokines and included the data within the multivariable linear regression models (Table 3, Supplementary Table 3). Probably in response to COVID-19 pneumonia and independently from vaccine administration, cytokines levels appeared overall strongly correlated with each other, in the three VAC groups (Fig. 3). No statistically significant differences were observed between vaccinated and not vaccinated patients (VAC_{2+3} vs. VAC_{no}). When considering the number of vaccination doses (Supplementary Table 3), we found higher levels of GM-CSF in VAC_2 vs. VAC_{no} ($p=0.0250$), meanwhile the pro-inflammatory cytokine IFN-α appeared to be reduced in VAC_3 vs. VAC_{no} ($p=0.0388$).

Both humoral and cellular immune response is influenced by the virus variants driving pneumonia

Overall, Delta variant was the most representative in VAC_2 ($n=13/18$, 72%), detected in 41% of VAC_0, whereases undetected in VAC_3 (Table 1). We then evaluated the impact of the type of variants (Delta vs. Omicron, Table 4) using a multiple regression adjusted for vaccine dose, age, gender and cancer. Delta infections were able

Table 3 Multiple regressions for comparison of groups VAC_{2+3} vs. VAC_{no} adjusted for age, gender and cancer. Tobit regression models for IgG-N (index), IgM-S (index) and IgG-RBD-S (BAU/mL). Linear regression models for the other outcomes

	VAC_{2+3} vs. VAC_{no}		
	Estimate	**Std.er.**	***p*-value**
IgG-N (index)	-1.6287	0.7961	0.0408
IgM-S (index)	0.2484	0.8002	0.7563
IgG-RBD-S (BAU/mL)	2.8312	0.9397	0.0026
IC50 229E	-0.304	0.342	0.381
IC50 HKU1	0.021	0.351	0.952
IC50 NL63	0.110	0.382	0.775
IC50 DELTA	0.685	0.311	0.034
IC50 BA1	1.982	0.949	0.044
IC50 BA4/5	0.678	0.315	0.038
GM-CSF pg/ml	0.527	0.484	0.284
IFN-α pg/ml	-0.391	0.366	0.294
IFN-γ pg/ml	0.036	0.339	0.915
IL-4 pg/ml	9.587	14.513	0.513
IL-5 pg/ml	0.291	0.350	0.411
IL-6 pg/ml	-0.068	0.350	0.848
IL-10 pg/ml	0.053	0.839	0.950
IL-12p70 pg/ml	0.132	0.498	0.792
IL-17 A pg/ml	0.150	0.518	0.774
TNF-α pg/ml	0.099	0.334	0.768
leukocytes	13.392	8.054	0.106
B cells count	700.739	233.339	0.005
B cells (% on CD45+)	1.651	0.637	0.014
B activated (CD19+/CD27+/IgD-/CD21-)	0.404	0.314	0.209
B resting (CD19+/CD27+/IgD-/CD21+)	-0.277	0.643	0.670
CD21low/CD38 low (CD19+/ $CD21^{low}$/$CD38^{low}$)	0.027	0.427	0.950
DN (CD19+/CD27-/IgD-)	0.076	0.461	0.871
Marginal Zone (MZ) (CD19+/CD27+/IgD+)	0.107	0.299	0.724
Memory B cells (MB) (CD19+/CD27+/IgD-)	-0.132	0.663	0.843
Naive B cells (CD19+/CD27-/IgD+/CD21-)	6.012	9.631	0.538
Plasmablast (CD19+/CD27+/IgM-/IgG-/$CD38^{high}$)	2.318	5.566	0.681
SWI (CD19+/CD27+/IgM-/IgG-)	-0.070	0.703	0.921
TLM (CD19+/CD27-/CD21-)	-0.123	0.482	0.800
Trans B (CD19+/CD27-/ $CD38^{high}$/$CD24^{high}$)	0.253	0.369	0.500
UNSWI (CD19+/CD27+/IgM+/IgG+)	-0.296	0.661	0.658
CD4 + T cells count	889.308	900.969	0.331
CD4+ (% of CD3+)	-2.434	7.447	0.746
CM-CD4 (CD4+/CD45RA-/CCR7+)	-4.022	4.497	0.378
N-CD4 (CD4+/ CD45RA+ /CCR7+)	-0.093	0.371	0.804
EM-CD4 (CD4+/ CD45RA-/ CCR7-)	1.461	5.256	0.783
TEMRA CD4 (CD4+/ CD45RA+/ CCR7+)	0.106	0.531	0.844
CD4+/PD1-/CD57-	119.221	456.438	0.796
CD4+/PD1-/CD57+	-0.072	0.271	0.791
CD4+/PD1+/CD57-	2.501	5.742	0.666
CD4+/PD1+/CD57+	0.085	0.563	0.880
CD4+/CD27-/CD28-	-0.281	0.546	0.610
CD4+/CD27-/CD28+	3.481	4.064	0.399
CD4+/CD27+/CD28-	0.259	0.377	0.498
CD4+/CD27+/CD28+	-2.927	5.969	0.627
CD8 + T cells count	1966.495	702.269	0.008
CD8+ (%of CD3+)	5.442	7.070	0.447
N-CD8 (CD8+/ CD45RA+ /CCR7+)	-0.247	0.231	0.294

Table 3 (continued)

	VAC_{2+3} vs. VAC_{no}		
	Estimate	**Std.er.**	***p*-value**
CM-CD8 (CD8+/CD45RA-/CCR7+)	-5.938	6.537	0.371
EM-CD8 (CD8+/ CD45RA-/ CCR7-)	3.330	5.416	0.543
TEMRA-CD8 (CD8+/ CD45RA+/ CCR7+)	0.496	0.677	0.469
CD8+/CD57-/PD1-	2.609	3.056	0.400
CD8+/CD57-/PD1+	-3.202	6.384	0.619
CD8+/CD57+/PD1-	0.128	0.701	0.857
CD8+/CD57+/PD1+	-2.653	5.087	0.606
CD8+/CD27-/CD28-	-1.105	7.444	0.883
CD8+/CD27-/CD28+	-0.402	0.271	0.148
CD8+/CD27+/CD28-	-0.745	1.936	0.703
CD8+/CD27+/CD28+	4.998	7.074	0.485
Th1 (CD4+/CCR6- /CXCR3+);	-3.974	4.653	0.400
Th17 (CD4+/CCR6+/ CCR4+)	-0.178	0.393	0.654
Th17-1 (CD4+/CCR6+/CXCR3+)	0.627	0.422	0.148
Th2 (CD4+/CCR6-/CCR4+)	0.975	0.351	0.009
Treg (CD4+/CD25+/$CD127^{low}$)	0.008	0.159	0.961

to elicit a higher humoral response in terms of IgM-S ($p=0.0301$) and IC50 vs. Delta ($p=0.0123$), with a trend for higher IgG-RBD-S ($p=0.0715$). Further, infections with Delta also increased pro-inflammatory cytokines, such as IFN-α ($p=0.0463$) and IL-6 ($p=0.0010$). Alongside a trend for higher IgG-RBD-S in Delta, we also observed an expansion in the B cells compartments, including resting B cells (CD27+IgD-CD21+, $p=0.0400$) and Switched B cells (CD27+IgD-IgM-, $p=0.0176$). Together with an increase of pentamer a-specific IgM-S in Delta infections, we reported higher levels of the naïve CD4 T cells ($p=0.0025$) and a decrease of the CD27- (memory) CD4 T cells ($p=0.0147$). Helper CD4 and CD8 populations did not appear to be affected by type of variants.

Discussion

In this work, we explored the humoral, cellular and soluble markers of immune response in aged patients with COVID-19 pneumonia caused by Delta/Omicron variants. We showed that vaccination supported an elevated anti-SARS-CoV-2 humoral and cellular response, regardless to age, gender or cancer history. The administration of three doses of vaccine, rather than two or none, was more frequent in elderly individuals above 70 years of age and was highly associated with less severe symptomatology and higher survival rate. The virus variants driving pneumonia played a central role in supporting both the cellular and humoral response. Our study provides comprehensive immunological profiling of a cohort of aged patients, which is unique because it does also include non-vaccinated subjects that were hospitalized for COVID-19 pneumonia.

A previous work explored the immunological features during COVID-19 pneumonia in a population of 33 vaccine naïve subjects above 70 years old compared to younger individuals (<60 years) [6]. The authors found that elderly population showed reduced capacity to mount a proper anti-viral response that could drive to more severe outcomes. In our cohort, we confirmed that older individuals who did not receive vaccination or only 2 doses experienced worse clinical outcome an higher probability of death. On the other hand, we confirmed that completion of the vaccine schedule (3 vaccination doses at the time of the study) was associated with an efficient immune response and milder clinical outcome, confirming general guidelines that seek to prioritise the elderly population for vaccination to avoid severe COVID-19 symptoms [15].

Patients within our cohort experienced respiratory distress syndrome (ARDS) caused by SARS-CoV-2 infection. All had a positive clinical outcome, excluding two patients over 70 years, one non-vaccinated and one that did not complete the vaccination schedule, who did not survive the infection, despite being initially assigned a score in the range of the study group. Despite the similar clinical course, we observed differences driven by the vaccination status (VAC_{no}, VAC_2, VAC_3,) that affected the immune responses during natural infection.

Vaccinated individuals also had elevated levels of Th2 cells, which are known to prevent immune-driven lung damage [35, 36]; this data is in line with others confirming the protective role of the vaccines towards worse clinical outcomes [37–40]. Whilst plasma cytokines appeared to be similar between vaccinated and non-vaccinated individuals, when stratifying the population according to the number of vaccine doses, we could make

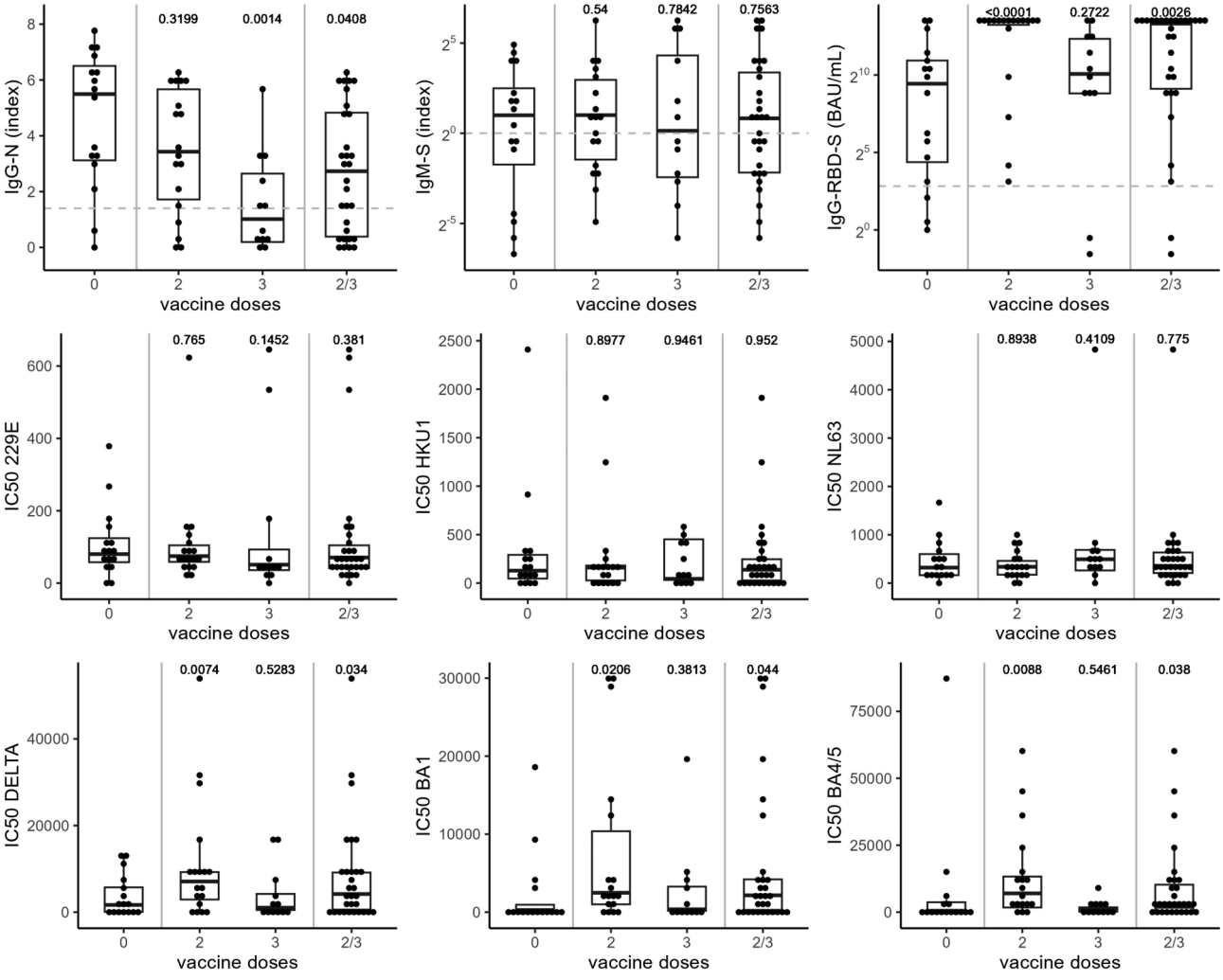

Fig. 1 Levels of SARS-CoV-2 specific antibodies and neutralizing activity. The dashed lines represent the cutoff values. p-values correspond to the comparison against the group VACno adjusted for age, gender and cancer. Tobit regression models for IgG-N (index), IgM-S (index) and IgG-RBD-S (BAU/mL). Linear regression models for the other outcomes. Full statistics report is available in Table 2 and Supplementary Table 2.

some observations. First, the levels of GM-CSF, which is known to be associated with virus clearance from lungs, [36] were significantly higher in VAC_2 but not in VAC_3 compared to VAC_{no}. Second, the levels of the pro-inflammatory cytokine IFN-α were significantly reduced in VAC_3 vs. VAC_{no}. As an expected consequence of the vaccination, VAC_{2+3} individuals showed higher anti-SARS-CoV-2 humoral response levels and expansion of B and CD8 cell populations, which appeared to be independent of age, gender or cancer history.

Neither vaccination nor variants driving infection had an impact on the neutralization activity vs. human seasonal coronaviruses. On the other hand, we observed differences in the anti-SARS-CoV2 humoral response when looking independently at VAC_2 and VAC_3 vs. VAC_{no}, which could be explained by the intrinsic variability of the group, vaccine doses or it could be attributed to the variants driving pneumonia, considering that majority of VAC_2 were infected with Delta virus and VAC_3 with Omicron virus. Thus, we explored whether virus variants driving pneumonia could impact the immune responses, including humoral, cellular and soluble markers. Compared to VAC_{no}, VAC_2 group (Delta infections) but not VAC_3 (Omicron infections) showed higher anti-SARS-CoV-2 IgG levels. Whilst this could be attributed to possible immune tolerance driven by multiple doses [14], we previously demonstrated that Delta viruses are associated with anti-RBD-IgG/neutralizing antibodies against Wuhan [34]. Our analysis confirmed that infections with Delta are not only capable of eliciting a higher immune humoral response, but they also support an increase in the B cell compartments (resting and switched).

In line with previous studies, we found that Omicron variant (mainly detected in VAC_3) had proportionally lower production of circulating anti-RBD-S IgG and higher levels of IgG-N antibodies. This could be a

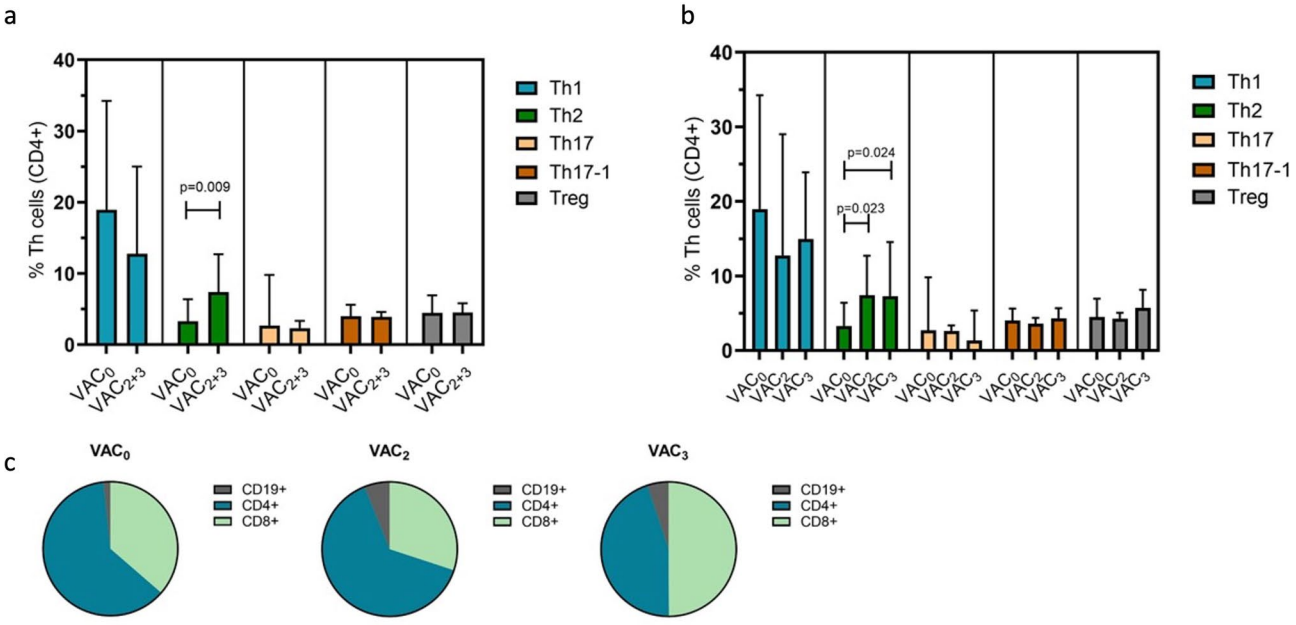

Fig. 2 The relative frequencies of T Helper subpopulation and Treg lymphocyte in the different groups of subjects. Bar plot representing the median and 95% IC of Th cells relative frequencies in non vaccinated (VAC0) or vaccinated (VAC2 + 3) (**a**) or depending on the number of doses (**b**). Pie-chart showing the relative frequency of CD19 + B cells, CD8 and CD4 T-cells sub-populations on CD3 + lymphocytes in VAC0, VAC2 and VAC3 (**c**). p-alues were obtained using non-parametric Spearman test. Levels of statistical significance was set at $p < 0.05$

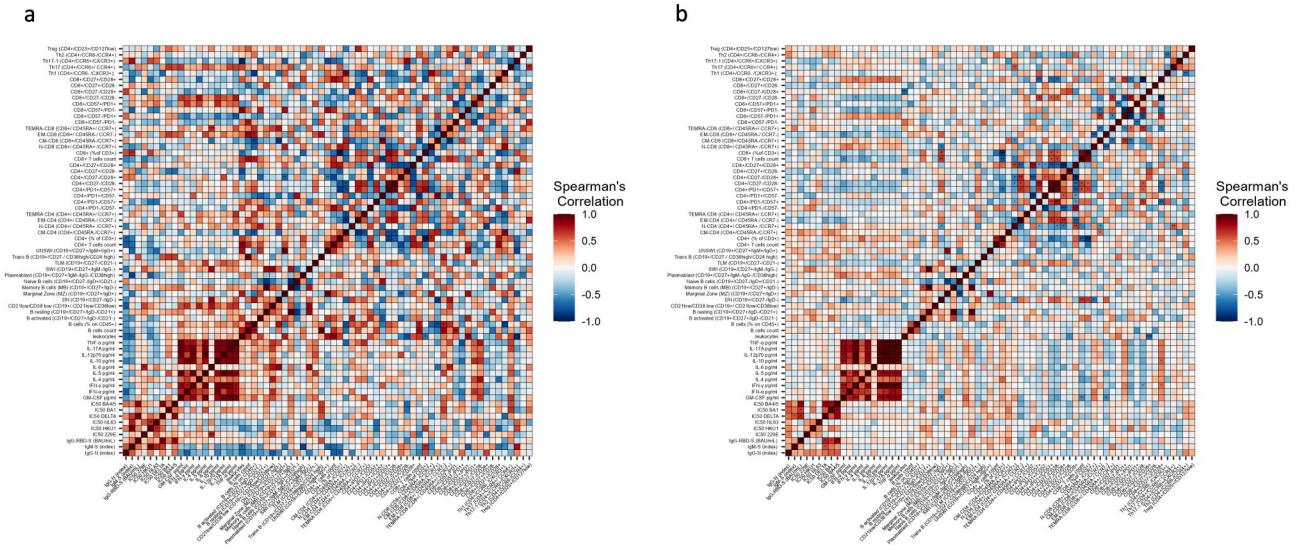

Fig. 3 Spearman's correlations between immunological responses in VACno ($n = 17$ in a) and VAC2 + 3 ($n = 30$ in b). The magnitude of each correlation is denoted with a colour, whereby the red colour indicates a positive correlation and the blue colour represents a negative correlation, such that the deeper the colour, the stronger the correlation. Levels of statistical significance with false discovery rate (FDR) correction are denoted as: $p < 0.05$, *$p < 0.01$, ***$p < 0.001$

reflection of the spike epitope immune escape mechanisms adopted by Omicron virus, which does also lead to an increment of CD8 T-cells (mainly cytotoxic) that we observed consistently with others [40, 41]. Consistently with the knowledge that Delta is more aggressive towards lung tissue than Omicron [42–44], we found that GM-CSF and IFN-α levels are higher in Delta vs. Omicron. Furthermore, whilst we reported above that variants could have an impact on the distribution of some cell populations, we observed that Th2 cells, which are associated with prevention from lung damage, were not affected by virus variants, but only a consequence of the vaccination, confirming again the protective role of vaccination against worse clinical outcome.

Table 4 Multiple regressions for comparison of Omicron and Delta, adjusted for vaccine doses, age, gender and cancer. Tobit regression models for IgG-N (index), IgM-S (index) and IgG-RBD-S (BAU/mL). Linear regression models for the other outcomes

	Delta vs. Omicron		
Outcome	Estimate	Std.er.	p-value
IgG-N (index)	0.6217	0.7509	0.4077
IgM-S (index)	1.5062	0.6943	0.0301
IgG-RBD-S (BAU/mL)	1.5130	0.8395	0.0715
IC50 229E	0.2817	0.3223	0.3879
IC50 HKU1	0.4711	0.3242	0.1551
IC50 NL63	0.3086	0.3594	0.3963
IC50 DELTA	0.7124	0.2698	0.0123
IC50 BA1	-0.5759	0.8979	0.5254
IC50 BA4/5	1.2422	0.9360	0.1931
GM-CSF pg/ml	0.4751	0.3420	0.1744
IFN-α pg/ml	0.6852	0.3305	0.0463
IFN-γ pg/ml	0.1774	0.3240	0.5879
IL-4 pg/ml	14.1453	13.7252	0.3105
IL-5 pg/ml	0.2850	0.3324	0.3976
IL-6 pg/ml	1.6181	0.4454	0.0010
IL-10 pg/ml	1.3452	0.7703	0.0903
IL-12p70 pg/ml	0.5763	0.4677	0.2269
IL-17 A pg/ml	0.8790	0.4731	0.0724
TNF-α pg/ml	0.3995	0.3129	0.2109
leukocytes	-2.9011	7.8949	0.7156
B cells count	269.8776	224.3362	0.2375
B cells (% on CD45+)	0.7588	0.6113	0.2232
B activated (CD19+/CD27+/IgD-/CD21-)	-0.0173	0.3028	0.9548
B resting (CD19+/CD27+/IgD-/CD21+)	0.9019	0.4162	0.0400
CD21low/CD38 low (CD19+/ $CD21^{low}$/$CD38^{low}$)	0.6866	0.3888	0.0896
DN (CD19+/CD27-/IgD-)	0.4834	0.4337	0.2757
Marginal Zone (MZ) (CD19+/CD27+/IgD+)	-0.1516	0.2872	0.6024
Memory B cells (MB) (CD19+/CD27+/IgD-)	0.6686	0.3927	0.1011
Naive B cells (CD19+/CD27-/IgD+/CD21-)	-7.1532	9.1820	0.4433
Plasmablast (CD19+/CD27+/IgM-/IgG-/$CD38^{high}$)	7.5426	5.1543	0.1558
SWI (CD19+/CD27+/IgM-/IgG-)	0.9232	0.3633	0.0176
TLM (CD19+/CD27-/CD21-)	-0.7770	0.4384	0.0885
Trans B (CD19+/CD27-/ $CD38^{high}$/$CD24^{high}$)	-0.0005	0.3564	0.9988
UNSWI (CD19+/CD27+/IgM+/IgG+)	-1.7455	4.7323	0.7154
CD4 + T cells count	786.8960	874.3323	0.3746
CD4+ (% of CD3+)	10.6719	7.0755	0.1410
CM-CD4 (CD4+/CD45RA-/CCR7+)	0.2753	4.3295	0.9497
N-CD4 (CD4+/ CD45RA+ /CCR7+)	13.4578	4.0624	0.0025
EM-CD4 (CD4+/ CD45RA-/ CCR7-)	-12.8855	4.4594	0.0072
TEMRA CD4 (CD4+/ CD45RA+/ CCR7+)	-0.2333	0.5096	0.6505
CD4+/PD1-/CD57-	14.5185	5.5581	0.0141
CD4+/PD1-/CD57+	-0.3390	0.2534	0.1913
CD4+/PD1+/CD57-	-9.2431	5.2551	0.0891
CD4+/PD1+/CD57+	-0.8698	0.5173	0.1034
CD4+/CD27-/CD28-	-0.9335	0.4966	0.0702
CD4+/CD27-/CD28+	-9.1499	3.5254	0.0147
CD4+/CD27+/CD28-	-0.3853	0.3559	0.2879
CD4+/CD27+/CD28+	15.2394	5.0018	0.0049
CD8 + T cells count	-1262.3090	653.8846	0.0622
CD8+ (%of CD3+)	-8.1591	6.7983	0.2386
N-CD8 (CD8+/ CD45RA+ /CCR7+)	0.0348	0.2200	0.8753

Table 4 (continued)

	Delta vs. Omicron		
CM-CD8 (CD8+/CD45RA-/CCR7+)	-8.3449	6.0393	0.1772
EM-CD8 (CD8+/ CD45RA-/ CCR7-)	0.5504	5.1589	0.9157
TEMRA-CD8 (CD8+/ CD45RA+/ CCR7+)	0.7101	0.6322	0.2703
CD8+/CD57-/PD1-	-1.1609	2.9039	0.6922
CD8+/CD57-/PD1+	1.2502	6.0777	0.8384
CD8+/CD57+/PD1-	-0.2581	0.6660	0.7011
CD8+/CD57+/PD1+	1.8455	4.8351	0.7054
CD8+/CD27-/CD28-	-5.2644	7.0267	0.4596
CD8+/CD27-/CD28+	-0.4830	0.2425	0.0555
CD8+/CD27+/CD28-	-1.2794	1.8300	0.4899
CD8+/CD27+/CD28+	9.8105	6.4971	0.1415
Th1 (CD4+/CCR6- /CXCR3+);	-1.9562	4.4188	0.6612
Th17 (CD4+/CCR6+/ CCR4+)	-0.1706	0.3736	0.6512
Th17-1 (CD4+/CCR6+/CXCR3+)	0.3190	0.3979	0.4290
Th2 (CD4+/CCR6-/CCR4+)	0.0029	0.3346	0.9931
Treg (CD4+/CD25+/CD127^{low})	-0.0103	0.1517	0.9463

This study presents some limitations that deserve discussion. First, the sample size was small and analysed cross-sectionally, thus subjected to casualties. Second, our study lacked a control group of vaccinated/non vaccinated subjects with COVID-19 mild disease without pneumonia, which could have helped to better define the impact of vaccination on preventing severe clinical outcome. Third, which is common to other similar studies, is the lack of clinical history before hospitalisation and thus the inability to accurately estimate timing of infection which can have an impact on the humoral response dynamic. Further and similarly to other studies, patients were treated with corticosteroids which may have an impact on the measured immune markers; however, administration was provided according to clinical practice to all patients and blood was collected after maximum 2 days. It is reasonable to think that the exposure to corticosteroids was similar in all patients and thus the putative impact of corticosteroids was negligible. Last point that deserves to be mentioned is that would have been interesting to explore in vitro activation towards SARS-CoV-2 specific peptides but considering that patients were treated with corticosteroids before sample collection, this approach was not feasible due to poor viability of the cells after resting.

Conclusions

The present study indicates that vaccination was protective of worse clinical outcome in individuals older than 70 years, that virus variants driving infection has a direct impact on the shape of the immune response and the set of data presented in this work can guide future studies on the impact of variants on the disease progression and outcome.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-024-05556-2.

Supplementary Material 1

Supplementary Material 2

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Author contributions

AR, CP supervised data collection and analysis and wrote the manuscript. LL designed the study, supervised data collection and analysis, and revised the manuscript. CS and NG performed the flow cytometry data, with the contribution of AM. CPa processed samples and contributed to data collection. FC and CB performed the statistical analysis, under the supervision of MSdS. TF, MMN, NT produced the neutralizing assays. ST and ER processing samples. CUF managed patients' recruitment and data collection. All authors provided input into the analysis and revised the manuscript.

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Data availability

Complete data set is available in Zenodo upon request.

Declarations

Ethics approval and consent to participate

The study was approved by San Raffaele Institutional Ethical Committee in date 14/04/2020, within the non-interventional study "ImmCOVID" and all patients were treated according to Institutional programs upon written informed consent.

Consent for publication
All authors have read and approved the manuscript.

Competing interests
Authors have no interests to declare.

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