



Safety and immunogenicity of mRNA-LNP COVID-19 vaccine CVnCoV in Latin American adults: A phase 2 randomized study



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ABSTRACT

Background: The COVID-19 vaccine candidate CVnCoV comprises sequence-optimized mRNA encoding SARS-CoV-2 S-protein encapsulated in lipid nanoparticles. In this phase 2a study, we assessed reactogenicity and immunogenicity of two or three doses in younger and older adults.

Methods: Younger (18–60 years) and older (>60 years) adults were enrolled in two sites in Panama and Peru to receive either 6 or 12 µg doses of CVnCoV or licensed control vaccines 28 days apart; subsets received a 12 µg booster dose on Day 57 or Day 180. Solicited adverse events (AE) were reported for 7 days and unsolicited AEs for 4 weeks after each vaccination, and serious AEs (SAE) throughout the study. Humoral immunogenicity was measured as neutralizing and receptor binding domain (RBD) IgG antibodies and cellular immunogenicity was assessed as CD4+/CD8 + T cell responses.

Results: A total of 668 participants were vaccinated (332 aged 18–60 years and 336 aged > 60 years) including 75 who received homologous booster doses. Vaccination was well tolerated with no vaccine-related SAEs. Solicited and unsolicited AEs were mainly mild to moderate and resolved spontaneously. Both age groups demonstrated robust immune responses as neutralizing antibodies or RBD-binding IgG, after two doses, with lower titers in the older age group than the younger adults. Neither group achieved levels observed in human convalescent sera (HCS), but did equal or surpass HCS levels following homologous booster doses. Following CVnCoV vaccination, robust SARS-CoV-2 S-protein-specific CD4 + T-cell responses were observed in both age groups with CD8 + T-cell responses in some individuals, consistent with observations in convalescing COVID-19 patients after natural infection.

Conclusions: We confirmed that two 12 µg doses of CVnCoV had an acceptable safety profile, and induced robust immune responses. Marked humoral immune responses to homologous boosters suggest two doses had induced immune memory.

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Abbreviations: SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; CVnCoV, CureVac mRNA-LNP COVID-19 vaccine; LNP, Lipid nanoparticles; CMI, Cellular mediated immunity.

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Background

In January 2020, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified as the agent responsible for the first cases of pneumonia with unknown etiology in Wuhan, China. Since then the COVID-19 pandemic has resulted in >230 million infected people and 4.7 million deaths globally [1]. There has

been an unprecedented research effort aimed at developing safe and effective human vaccines against this virus and the World Health Organization (WHO) currently notes 117 vaccines in different phases of clinical development and 194 in preclinical stages [2] as part of an ongoing effort to meet the global need for vaccine supply. The antigenic target for the majority of these vaccines is the SARS-CoV-2 spike protein (S-protein). Neutralizing antibodies against S-protein have been shown to protect against SARS-CoV-2 infection in preclinical models [3]. Several different approaches have been used in vaccine development including one novel platform, which is the use of mRNA coding for S-protein as the vaccine antigen [4–6]. The CureVac vaccine candidate CVnCoV is a sequence-optimized mRNA, developed using the proprietary RNActive® technology platform. The mRNA, which encodes a form of S-protein from the wild-type strain that includes two proline mutations (S-2P) previously shown to stabilize the conformation of the S proteins for MERS-CoV [7] and SARS-CoV [8], is encapsulated in lipid nanoparticles (LNP).

In a phase 1 study, we demonstrated the safety, acceptable tolerability and immunogenicity of different dosages of CVnCoV when administered in a two-dose series four weeks apart to healthy 18–60 year-old adults [9]. A dose of 12 µg was found to be optimal in terms of balancing reactogenicity and immunogenicity, with a range of neutralizing antibody titers that overlapped those found in sera of convalescent COVID-19 patients. As the burden, notably mortality, of COVID-19 increases with age [10] we wanted to ensure that CVnCoV is safe and immunogenic in older adults who may display lower responses due to immunosenescence. The current phase 2a study was performed to assess the safety and immunogenicity of two 12 µg CVnCoV doses to confirm that the final dose of 12 µg is suitable for adults over 60 years of age. The 18–60 year-old cohort was included to bridge the current study to the phase 1 trial. Active control groups received licensed pneumococcal vaccine in the older adults, and licensed hepatitis A vaccine in the younger adults. While analyzing this study the overall vaccine efficacy of CVnCoV against symptomatic disease were estimated at 48.2% (95% CI: 31.0–61.4) in a major phase 2b/3 efficacy study (HERALD) involving 40,000 adult participants [11]. With this moderate efficacy and the ongoing emergence of SARS-CoV-2 variants development of the CVnCoV candidate has ceased to focus on the clinical development of a second generation vaccine candidate, CV2CoV. To contribute to that development we also investigated the impact of a third “booster” dose of CVnCoV given either four weeks after the second priming dose in a subset of older adults, or five months later in subsets of younger and older adults. This interim report describes the safety and reactogenicity data to 6 weeks after the second vaccination, and immunogenicity results in all groups. In addition, we report on immunogenicity results up to one month after receiving booster vaccinations.

Methods

This phase 2a active-controlled study with blinded and open-label phases is ongoing at two sites, the Centro de Vacunación Internacional (CEVAXIN), Panama City, Panama, and the Instituto de Investigación Nutricional, Lima, Peru. The protocol was approved by the appropriate institutional and national ethics committees, and was registered at [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT04515147) NCT04515147. The trial is being performed according to ICH E6 and Good Clinical Practice guidelines. All participants provided written informed consent at enrollment. The study is being overseen by an internal Safety Review Committee (iSRC) and an independent Data Safety Monitoring Board (DSMB) composed of external experts.

The co-primary objectives are to confirm the safety, in comparison with age-appropriate licensed non-COVID-19 vaccines, and

the immunogenicity of two 12 µg doses of CVnCoV given four weeks apart to older adults (>60 years of age) and young adults (18–60 years). Secondary objectives include the immunogenicity of a 12 µg booster dose administered to subsets of older adults at Day 57 days post first vaccination, or subsets of younger and older adults 180 days after the first vaccination, and the assessment of safety and reactogenicity after all doses. Exploratory objectives include assessments of the cell mediated immune (CMI) responses to vaccination. As this was the first use of CVnCoV in Latin American adults and in adults over 60 years of age two small sentinel groups with the 6 µg dose were assessed first.

Participants and inclusion/exclusion criteria

Participants are males or non-pregnant females 18 years of age or older, in good general health following a physical examination and laboratory assessments, or with chronic health conditions considered to be well controlled with treatment in the opinion of the investigator. Inclusion criteria included a body mass index ≥ 18.0 and ≤ 32.0 kg/m², compliance with protocol procedures and availability for clinical follow-up to the last planned visit. Volunteers were recruited independently of their SARS-CoV-2 serostatus, which was determined retrospectively by RT-PCR testing of nasopharyngeal swabs taken at enrollment and by ELISA for the SARS-CoV-2 N-antigen (EI 2606–9601-2 G, EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany) at baseline and at each time point. Since the vaccine does not contain the N-antigen this allowed for per protocol analyses in initially seronegative participants and post-hoc analyses seropositive.

Once enrolled volunteers were randomized 10:1 using a sponsor-supplied electronic randomization schedule (IRT) prepared by the CRO to receive either the selected dose of CVnCoV or the active control in an observer-blinded manner.

Female participants of childbearing potential were to have a negative pregnancy test (blood hCG) on the day of enrollment and had to agree to use an approved birth control method from two weeks before the first vaccination until three months after the last administration. The main exclusion criteria included use of any investigational or non-registered product (including other COVID-19 vaccines) or other vaccine from 28 days before the first dose of trial vaccine and throughout the trial period, any treatment with immunosuppressants or other immune-modifying drugs within 6 months prior to the administration of the trial vaccine or planned use during the trial, with the exception of topically-applied, inhaled, or intranasal steroids, any diagnosed or suspected immunosuppressive or immunodeficient condition including known HIV, HBV or HCV infection, any history of immune-mediated or autoimmune disease or anaphylaxis or allergy to any component of CVnCoV or aminoglycoside antibiotics. Also excluded were individuals who had been active smokers within the last year (including any vaping), and those who had a history of virologically-confirmed SARS, MERS, or COVID-19 disease or known exposure (without any personal protective equipment) to an individual with confirmed COVID-19 disease or SARS-CoV-2 infection within the past 2 weeks, or anyone the investigator considered to be at increased risk of exposure to COVID-19 disease.

Vaccine

The CVnCoV vaccine candidate is an LNP-formulated RNActive® SARS-CoV-2 vaccine that contains 6 or 12 µg mRNA encoding for a pre-fusion conformation-stabilized version of the full-length S-protein from wild-type SARS-CoV-2. The mRNA is encapsulated in four lipid components: cholesterol, 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), PEG-ylated lipid and a cationic lipid [12], and is stored at –60°C until use. Each 0.3 mL dose was admin-

istered by intramuscular injection in the deltoid muscle of the non-dominant arm. Age-appropriate control vaccines were a licensed hepatitis A vaccine (Havrix™, GSK, Rixensart, Belgium, lot AHAVB965BK in Panama; and Avaxim™, Sanofi Pasteur, Lyon, France, lot R3E148V in Peru) which is recommended for use in the 18–60 years groups, and a licensed pneumococcal vaccine (Prevenar13, Pfizer, Ireland; lots AN1061 and T019826 in Panama, and lot DP8378 in Peru) which is recommended for the over-60 participants, administered according to the manufacturers' instructions.

Procedures

Volunteers were sequentially enrolled initially to the 6 µg cohort (Group 1: 18–60 years [n = 12] and Group 2: > 60 years [n = 11]), then into the 12 µg cohort (Group 3: 18–60 years [n = 90] and Group 4: > 60 years [n = 90]) for which enrollment only began once the 6 µg groups were complete. Vaccine groups and active controls (Group 5: 18–60 years [n = 9] and Group 6: > 60 years [n = 9]) were enrolled in parallel and randomized 10:1. The first four participants enrolled in Group 2 were sequentially vaccinated at least one hour apart with a 6 µg dose and safety data were recorded for 24 h. The iSRC assessed these data, particularly any Grade 3 adverse events, before giving approval for the remaining participants in the group to be vaccinated. This process was repeated with the first 12 participants enrolled into Group 4 with the 12 µg dose. Following confirmation of the final dose selection two expansion cohorts of 220 participants in each age group were enrolled, randomized 10:1 to receive either the selected dose of CVnCoV or the respective active control vaccine in an observer-blinded manner.

Unblinded study staff, with no role in data collection for safety or immunogenicity assessments, administered the first vaccinations on Day 1 and the second on Day 29. Two subsets of Group 4 participants, from the first participants enrolled in each of the two countries, received an open-label 12 µg booster dose on Day 57 (n = 30) or Day 180 (n = 15), and a subset of Group 3 participants received a 12 µg booster dose on Day 180 (n = 30).

Safety

Participants were monitored for 30 min after vaccination and then they recorded, on a daily basis, the occurrence and severity of solicited local (injection site pain, redness, swelling, and itching) and systemic adverse events (AEs; headache, fatigue, chills, myalgia, arthralgia, nausea/vomiting, and diarrhea), and oral temperature for 7 days in diaries. Severity was graded as 0: absent, 1: mild, 2: moderate or 3: severe according to the criteria in *Supplementary table 1*. Occurrence of any unsolicited AE during the 28 days after each vaccination was also recorded. Serious AEs (SAE) or AEs of special interest (AESI) using the definitions of the Brighton Collaboration via CEPI's Safety Platform for Emergency vACcines [SPEAC] project [13] were to be reported immediately to the investigator throughout the duration of the study. The investigator assessed the relationship of each systemic AE, SAE or AESI to the trial procedures. Blood samples were taken on Days 1, 2 and 29 and subsequent visits if abnormal values were detected for determination of hematology (complete blood count, including differential and platelets), clinical biochemistry, and coagulation and graded according to the United States Food and Drug Administration (FDA) toxicity grading scale [14]. All participants were tested using a reverse transcription polymerase chain reaction (RT-PCR) for SARS-CoV-2 at baseline, and those who presented with symptoms indicative of COVID-19 infection, i.e. cough, shortness of breath, difficulty breathing, fever ≥ 37.8 °C, fatigue, myalgia, chills, wheezing, nasal congestion, runny nose, sore throat,

headache, diarrhea, or new olfactory or taste disorders, during the study were also tested. In the case of any of these symptoms occurring the investigator arranged for the RT-PCR as soon as possible and followed up with repeat testing 7 to 14 days later.

Humoral immunogenicity

Sera were obtained before vaccination on Days 1 and 29 (and Days 57 and 180 for booster subsets), and postvaccination on Day 43 (and Days 85, 180 and 208 for booster subsets). Immune responses were assessed as SARS-CoV-2 virus 50% neutralization titers determined by a microneutralization assay (MNT₅₀) and as IgG-antibodies binding to the receptor binding domain (RBD) of the S-protein measured by ELISA as previously described [9]. Both assays were performed at Vismederi Srl (Siena, Italy) and had been validated in accordance with EMA, FDA and ICH guidance. The MNT assay was calibrated with the 1st WHO International Standard anti-SARS-CoV-2 immunoglobulin, human developed by the National Institute for Biological Standards and Controls (NIBSC code 20/136), and MNT₅₀ titers can be converted to International Units per milliliter (IU/mL) by multiplying by a factor of 1.704. In the expansion phase MNT was only assessed in a subset of participants from the site where booster doses were administered. Results are presented as group geometric mean titers (GMT) at each time point for the immunogenicity set, defined as those who were seronegative at baseline and did not display any indication during the study from NAAT testing or anti-N protein serology. For comparison a panel of previously described [9] human convalescent sera (HCS) obtained from 68 convalescent patients mainly 4–8 weeks after symptomatic COVID-19 illness, aged 18–74 years, were tested for antibodies in the same assays.

Cellular immunogenicity

Subsets of the first 20 participants who received the 12 µg dose in both age groups were to be assessed for CMI. At the time of this report not all samples were available from those subsets so the analysis was done on the samples already obtained. Peripheral blood mononuclear cells (PBMC) were obtained from these subsets at Days 1, 29 and 43 and stored in liquid nitrogen. They were analyzed at the CEVAC laboratory (Ghent, Belgium) using a flow cytometry-based 8-parameter T cell intracellular cytokine staining (ICS) assay [15,16]. The assay focused on a Th1 response and included staining for lineage markers, CD3+, CD4 + and CD8 + T cells, and a panel of functional markers, CD40L, IFN-γ, TNF-α, and IL-2 after stimulation of PBMCs with two peptide pools, Sp1 and Sp2 (15mers with 11 amino-acid overlap), which together span the entire sequence of the SARS-CoV-2 S-protein (see *Supplementary material* page 3).

Statistics

In this exploratory trial with no hypothesis testing, only descriptive statistics were used unless otherwise stated. The sample size is considered adequate to ensure sufficient safety and immunogenicity data are available to make decisions on dose and schedule selection to be applied in phase 3 clinical trials. In the initial part of the study 220 participants (200 vaccinees and 20 controls) were to be enrolled, and in a planned expansion phase a further 440 (400 vaccinees and 40 controls) were recruited giving the totals in Groups 3 and 4 shown in the flow chart (Fig. 1).

Geometric mean titers (GMTs) with 95% confidence intervals were calculated for RBD-binding IgG antibodies and SARS-CoV-2-neutralizing antibodies summarized according to CVnCoV dose level, age group, baseline serostatus and time point. Values were converted to log base 10, the arithmetic mean and 95% CI margins

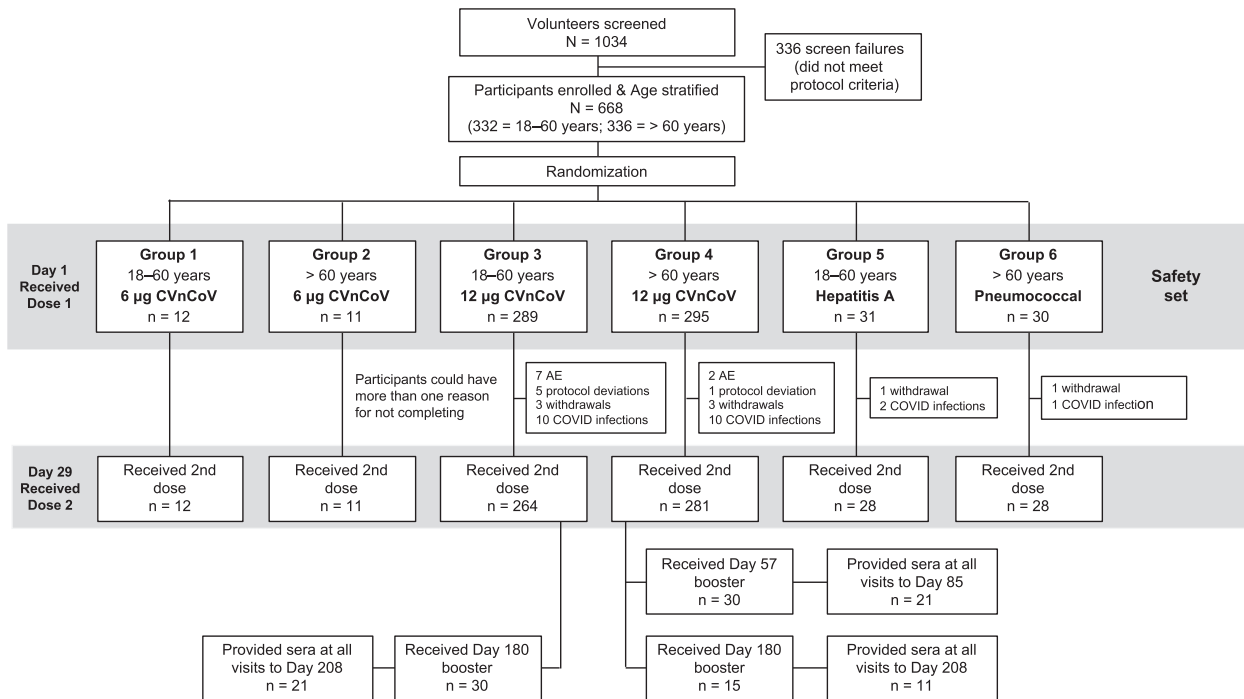


Fig. 1. Study flow chart.

calculated from these log-transformed values and afterwards antilog-transformed for geometric mean with the 95% CI. Group seroconversion rates for RBD-binding antibodies and SARS-CoV-2-neutralizing antibodies were calculated in those who were seronegative at baseline, defined as at least a four-fold increase in titer over baseline. Group geometric mean-fold rise (GMFR) in titers post-vaccination were calculated either to baseline (for Day 29 and 43 values after one and two doses) or to the titer values from the day of booster vaccination as depicted in Supplementary table 3.

Statistical analysis of vaccine-induced SARS-CoV-2 S-protein-specific CD4 + and CD8 + T cell responses was performed using a Wilcoxon matched-pairs signed rank test and the results of Day 29 and Day 43 compared with Day 1 by age group and PBMC stimulation (Sp1 and Sp2, and Sp1 + Sp2). Statistical significance was set at a p-value of ≤ 0.05.

Results

The study started on 21 September 2020 with a database lock for the safety data analyses on 19 March 2021. This was six weeks after receipt of the second vaccination in 624 participants; totals of 332 adults aged 18–60 years and 336 aged > 60 years were enrolled and randomized to the respective study groups as shown in Fig. 1, including 30 participants who received a booster dose at Day 57. Additional safety and reactogenicity data was collected in the subsets after booster doses at Day 180. The immune response was determined four weeks after the second dose in all participants, and after the booster dose in the subsets of participants who received boosters. Enrollment was distributed equally in Peru (153 18–60 year-olds and 182 > 60 year-olds) and Panama (179 18–60 year-olds and 154 > 60 year-olds). Overall compliance with

Table 1 Demographics of the enrolled study population (Safety Set) by group.

Group		1	2	3	4	5	6
Vaccine		6 µg CVnCoV		12 µg CVnCoV		Hepatitis A	Pneumococcal
Age group		18–60 years	> 60 years	18–60 years	> 60 years	18–60 years	> 60 years
Age (yrs)	N=	12	11	289	295	31	30
	Mean	50.2	65.6	38.16	69.0	31.6	66.7
	SD	8.4	4.1	12.1	6.1	12.3	5.0
	range	(34–60)	(61–73)	(28–48)	(64–73)	(21–41)	(63–71)
Male	n (%)	5 (42)	7 (64)	172 (59.5)	157 (53.2)	19 (61)	14 (47)
Female	n (%)	7 (58)	4 (36)	117 (40.5)	138 (46.8)	12 (39)	16 (53)
BMI (kg/m ²)	Mean	26.9	26.3	26.3	26.3	25.9	25.4
	SD	(1.49)	(2.28)	(3.29)	(3.16)	(3.94)	(3.32)
Ethnicity	n (%)						
	Hispanic or Latino	12 (100)	11 (100)	283 (97.9)	287 (97.3)	31 (100)	30 (100)
Not Hispanic or Latino		0	0	6 (2.1)	8 (2.7)	0	0
Serology*	n (%)						
	Seropositive	0	1 (9)	29 (10.0)	27 (9.2)	3 (10)	2 (7)
	Seronegative	12 (100)	10 (91)	249 (86.2)	161 (54.6)	23 (74)	17 (57)
	Unknown as analysis incomplete	0	0	11 (3.8)	107 (36.3)	5 (16)	11 (37)

* Based on retrospective assessment of baseline SARS-CoV-2 IgG N-antigen by ELISA.

study procedures was good, with a low rate of drop-outs that were not due to symptomatic COVID-19 infection. The demographics across the different study groups were similar in the two age cohorts (Table 1). At the time of this report, the retrospective serology to determine prior exposure to SARS-CoV-2 was not available for 134 participants included in the safety analyses, but in those with data, the balance of baseline seropositives vs. seronegatives (approximately 10% vs 90%) was similar across groups.

Safety

Vaccination was generally safe and well-tolerated. There were no deaths, and none of the ten SAEs reported, nine in recipients of 12 µg doses of CVnCoV and one after a pneumococcal vaccination (Table 2), were considered to be related to the vaccination

by the investigators. Of 45 participants with AESIs, 37 in the 12 µg group and 8 active controls, only one, an increase in blood pressure, was considered to be related to vaccination. The other AESIs were all COVID-19 infections (36 and 8 in the 12 µg CVnCoV and active control groups, respectively) including two cases of COVID-19 pneumonia in 12 µg CVnCoV recipients. Similar proportions of the 12 µg CVnCoV and active control groups had AEs that led to vaccine withdrawal. The four withdrawals from the study (three for mild COVID-19 symptoms, one for anxiety) were in the 12 µg CVnCoV group. The proportion of individuals requiring medical attention for an AE was higher in the active controls than the 12 µg CVnCoV group (Table 2).

The incidence of solicited local AEs after either 12 µg dose of CVnCoV was higher in the 18–60 year-olds than the over-60 year-olds, 87.5% vs 74.6% after the first dose and 76.5% vs

Table 2 Solicited AEs after first and second vaccinations, and overall unsolicited reactogenicity and other adverse events by study group.

	Group	1		2		3		4		5		6	
		Vaccine		6 µg CVnCoV		12 µg CVnCoV		Hepatitis A		Pneumococcal			
		Age group		18–60 years	> 60 years	18–60 years	> 60 years	18–60 years	> 60 years	18–60 years	> 60 years	18–60 years	> 60 years
Solicited AE – dose 1	N =	12		11		289		295		31		30	
	Local	9 (75.0)		5 (45.5)		253 (87.5)		220 (74.6)		17 (54.8)		26 (86.7)	
	Systemic	5 (41.7)		6 (54.5)		227 (78.5)		193 (65.4)		13 (41.9)		15 (50.0)	
Solicited AE – dose 2	N =	12		11		264		281		28		28	
	Local	9 (75.0)		3 (27.3)		202 (76.5)		180 (64.1)		6 (21.4)		21 (75.0)	
	Systemic	9 (75.0)		8 (72.7)		212 (80.3)		192 (68.3)		13 (46.4)		10 (35.7)	
Grade 3 AEs	Any	0		2 (18.2)		28 (9.7)		19 (6.4)		0		0	
	Related	0		2 (18.2)		21 (7.3)		16 (5.4)		0		0	
Unsolicited AE	Any	7 (58.3)		6 (54.5)		166 (57.4)		156 (52.9)		17 (54.8)		12 (40)	
	Related	3 (25)		3 (27.3)		69 (23.9)		54 (18.3)		3 (9.7)		4 (13.3)	
SAE	Any	0		0		6 (2.1)		3 (1.0)		0		1 (3.3)	
	Related	0		0		0		0		0		0	
Medically attended AEs	Any	3 (25)		3 (27.3)		34 (11.8)		34 (11.5)		6 (19.4)		4 (13.3)	
	Related	0		2 (18.2)		12 (4.2)		10 (3.4)		2 (6.5)		1 (3.3)	
Any AE leading to vaccine withdrawal		0		0		21 (7.3)		12 (4.1)		3 (9.7)		1 (3.3)	
Any AE leading to withdrawal from study		0		0		3 (1.0)		1 (0.3)		0		0	
Any AESI	Any	0		0		21 (7.3)		16 (5.4)		6 (19.4)		2 (6.7)	
	Related	0		0		0		1 (0.3)		0		0	

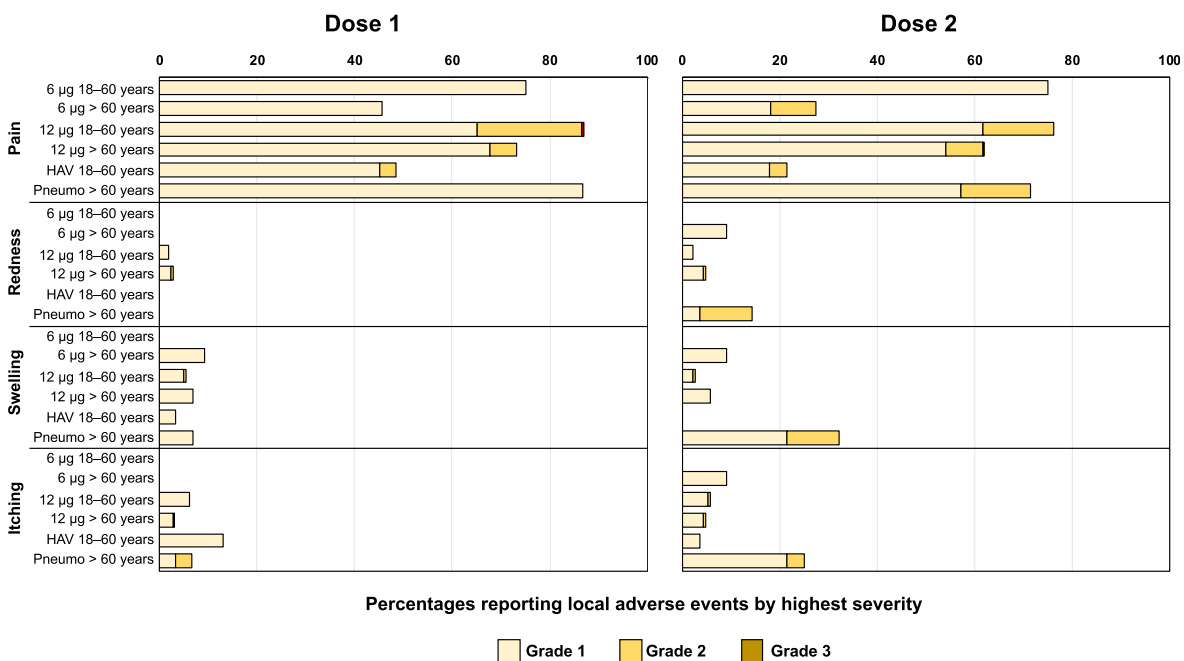


Fig. 2. Local reactogenicity with severity in all groups in the 7 days after the first and second vaccinations.

64.1% after the second. Rates after pneumococcal vaccine in over-60 year-olds were similar to those in 18–60 year-olds 12 µg CVnCoV, the lowest rates being observed in the 18–60 year-olds who received Hepatitis A vaccine (Table 2). The majority of local AEs were pain at the injection site (Fig. 2), reported after 86.9% of first doses, and 76.1% of second doses in the 18–60 year-old 12 µg group. Most of these were mild or moderate, with only one report of Grade 3 pain. The older adults reported pain after 73.2% and 61.9% of first and second doses respectively, with one report of Grade 3 pain. Occurrence of other local AEs was rare, the most frequent and highest severity being in the older adults given pneumococcal vaccine. Most local AEs started within 24–72 h of vaccination and resolved within 1 or 2 days; the two Grade 3 cases resolved within 1 day.

There were small age-dependent differences in the proportions of participants reporting solicited systemic AEs after 12 µg CVnCoV which were reported more frequently by the younger adults (Table 2). Similar proportions reported systemic AEs after first and second 12 µg doses. There were more reports after the second of the 6 µg doses than the first. Second doses were also associated with an increase in severity for some AEs (Fig. 3), most notably in the older adults who received the 6 µg dose. The most frequent AEs after the first and second doses were headache, fatigue, and myalgia in 12 µg CVnCoV and active control groups. Grade 3 solicited AEs were not reported in any control participant, but three were reported by two of the older 6 µg CVnCoV recipients (chills and myalgia). In the 12 µg CVnCoV groups 29 (5.0%) of 584 participants reported grade 3 solicited adverse events after any dose. Totals of

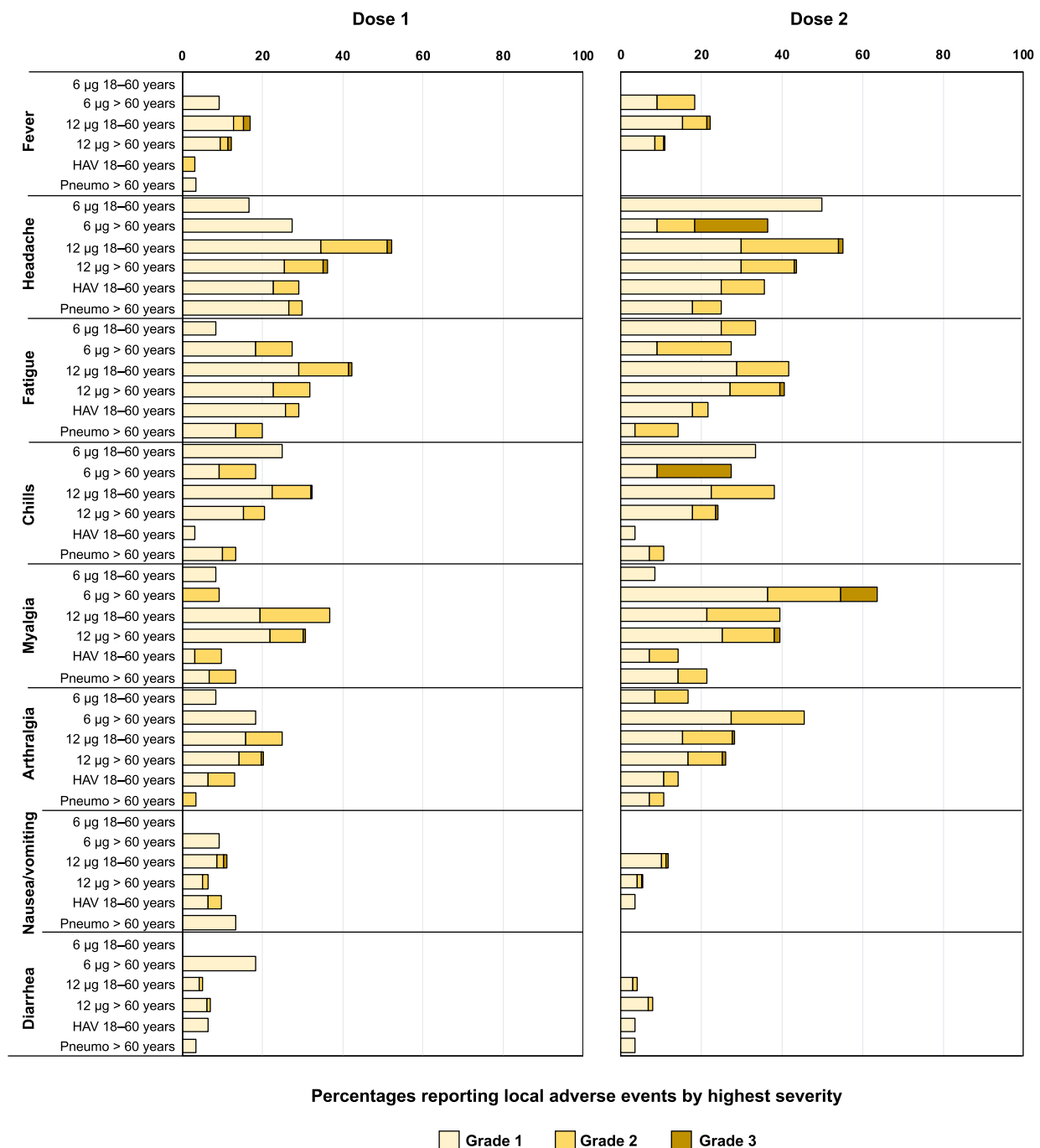


Fig. 3. Solicited systemic reactogenicity with severity in all groups in the 7 days after the first and second vaccinations.

grade 3 solicited and unsolicited adverse events are shown in Table 2. Mean duration of solicited systemic AEs in the 12 µg groups was 1.2 ± 0.45 days and 1.5 ± 0.71 days in the active controls. The median duration of Grade 3 AEs was 1 day before they resolved or presented as Grade 1 or 2.

Solicited reactogenicity after the Day 57 booster dose in older adults was less frequent and less severe than after the two previous doses (Supplementary table 2). All local reactions were described as mild or moderate, the most frequent being injection site pain, reported by 15 (50%) of the 30 participants,

two and three cases of mild redness and swelling, respectively. Half the participants reported a solicited systemic AE, all of which were also mild or moderate, the most frequent being fatigue, myalgia and headache. Median duration of all solicited AEs after the booster was 2 days. Reactogenicity to Day 180 boosters was similar to that observed after the first dose in both age groups (Supplementary table 2), most local reactions being mild-to-moderate injection site pain and the most frequent solicited adverse events being mild-to-moderate headache, myalgia and fatigue.

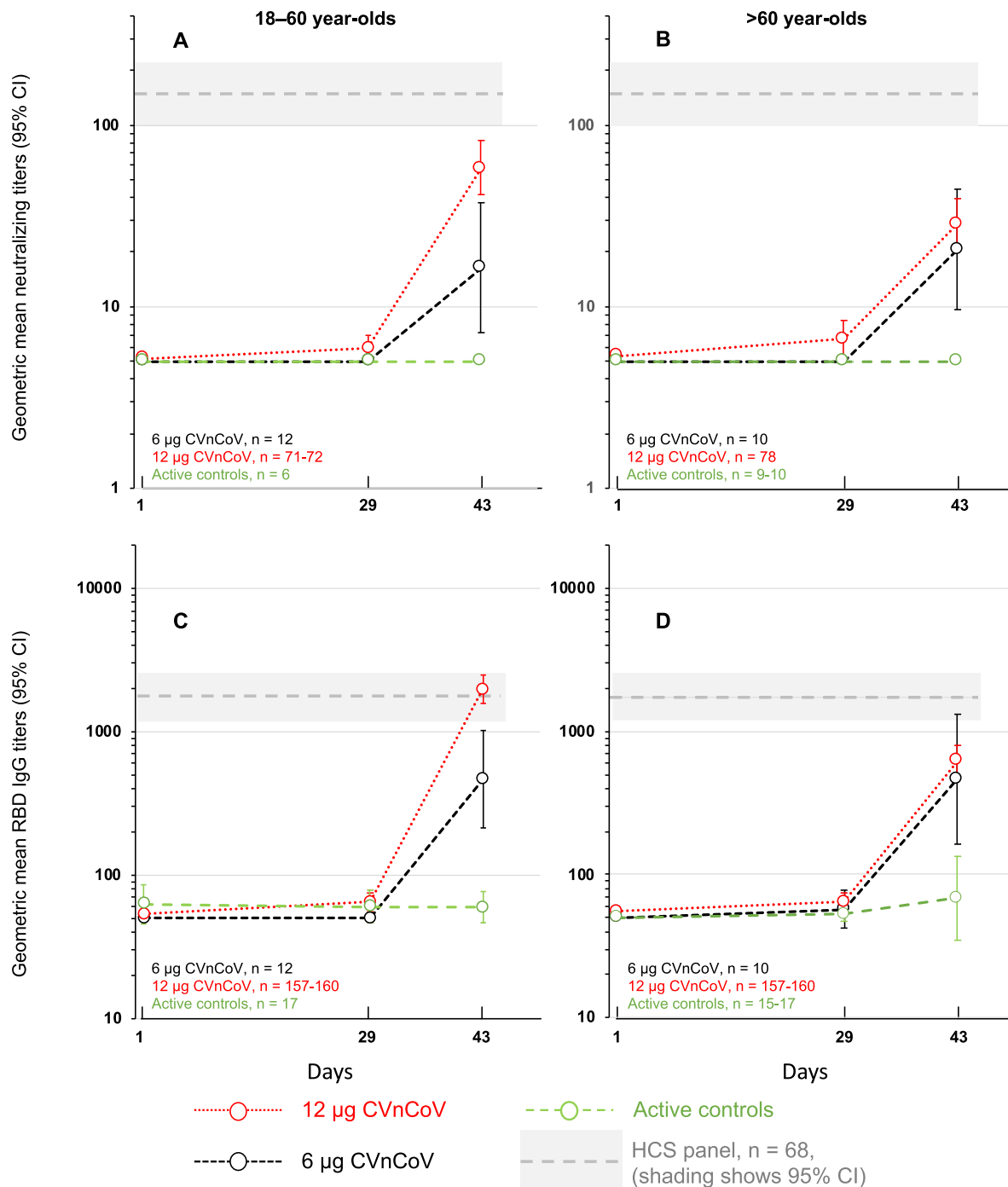


Fig. 4. Geometric mean titers (95% CI) of neutralizing antibodies (panels A & B) and receptor binding domain (RBD) IgG antibodies (panels C & D) following vaccination with 6 µg or 12 µg CVnCoV or active control vaccines on Days 1 and 29 in seronegative younger (18–60 years; panels A and C) and older adults (>60 years; panels B and D) who received two doses. Lowest value considered as positive is 10 for neutralizing titers and 100 for RBD IgG titers. Grey dashed lines show the respective GMTs of the panel of 68 human convalescent sera (HCS), shading shows 95% confidence interval.

Humoral immunogenicity

In the CVnCoV vaccine groups, there were increases in titers and seroconversion of SARS-CoV-2 neutralizing antibodies in some individuals on Day 29, one month after the first dose of 12 µg CVnCoV, which were not evident in the overall group GMTs (Fig. 4 A & B, Table 3). Marked increases were observed by Day 43, two weeks after the second doses, when some of the 6 µg recipients also responded. The GMT increases were dose-dependent in the 18–60 year-olds (Fig. 4A). At 58.2 MNT₅₀ (95% CI: 41.4, 81.9), the peak GMT at Day 43 in the younger adults given 12 µg CVnCoV was lower than the HCS panel of sera from convalescent COVID-19 patients (147 MNT₅₀ [98.5, 220]), and was higher than the 28.4 MNT₅₀ (20.6, 39.2) observed in the older adults given 12 µg CVnCoV. Neither of the active control groups displayed any consistent increase in SARS-CoV-2 neutralizing activity levels in those who were initially seronegative at baseline.

In younger participants who displayed no prior SARS-CoV-2 infection at baseline (seronegative for N-antigen) or during the study Day 43 neutralizing seroconversion rates were 41.7% and 80.3% and GMFR of 3.3 and 11.3 after two 6 µg or 12 µg CVnCoV doses, respectively (Table 3, Supplementary table 3). For seronegative over-60 year-olds (Fig. 4B) the neutralizing seroconversion rates (70.0% and 60.3%) and GMFR (4.1 and 5.3) were similar for the 6 µg or 12 µg CVnCoV doses (Table 3, Supplementary table 3).

In participants in both age groups who were seropositive at baseline before receiving two 12 µg CVnCoV doses, the confidence intervals of neutralizing GMTs at Day 29 overlapped the range of the HCS panel. Highest GMTs were observed in younger adults (Fig. 5A and 6). Unlike participants who were seronegative at base-

line, those who were seropositive demonstrated increases in neutralizing titers that achieved GMTs close (older participants) or higher (young adults) than the HCS panel at Day 29 after receiving one 12 µg dose of CVnCoV, and a further increase at Day 43 after the second dose (Fig. 5A and B).

The patterns of RBD-binding IgG, assessed by ELISA, were similar to the neutralizing responses, with dose-dependent increases in RBD IgG titers after the second 12 µg CVnCoV dose in 18–60 year-olds to the same level observed in the HCS at Day 43 (Fig. 4C). There was no dose-dependence in the older adults, in whom two 6 µg or 12 µg CVnCoV doses induced similar GMTs that remained lower than the range observed in the HCS panel (Fig. 4D). This was confirmed by the seroconversion rates and GMFR for the different dose and age groups (Table 3, Supplementary table 3). This age-dependent RBD-binding IgG response to the 12 µg dose was also apparent when participants were categorized according to their baseline serostatus, both seropositive age groups achieving higher GMTs than the HCS panel by Day 43 (Fig. 5B).

Full immunogenicity data were available from two subsets of older adults who were seronegative at baseline, and received a booster dose on either Day 57 (n = 21) or a booster on Day 180 (n = 11). There were small declines in GMTs of neutralizing antibodies and RBD-binding IgG from Day 43 to Day 57 in the first subset (Fig. 6), but four weeks (Day 85) after the booster dose, GMTs for both neutralizing and RBD-binding IgG antibodies had increased to levels overlapping those of the HCS panel. Seroconversion rates for neutralizing and RBD IgG antibodies increased to 95.2% at Day 85, with GMFR from Day 57 of 2.6 and 4.6, respectively (Table 3, Supplementary table 3). In the second subset of older adults, before the booster on Day 180, neutralizing and RBD-

Table 3

Seroconversion rates to baseline (Day 1) of neutralizing antibodies and IgG antibodies to RBD in the study groups after first, second and booster vaccinations. Seroconversion rate at Day 85 after booster at Day 57; seroconversion rate at Day 208 after booster at Day 180. Assayed in the immunogenicity set of participants who were seronegative at baseline and showed no evidence of infection with positive NAAT testing or anti-N-protein serology during the study).

Age (years)	Vaccine	1		2		3		4		5		6	
		6 µg CVnCoV		12 µg CVnCoV		HAV [#]		Pneumo [#]					
		18–60	> 60	18–60	> 60	18–60	> 60	18–60	> 60	18–60	> 60	18–60	> 60
Neutralizing antibodies													
Seroconversion ^a , n/N (%)													
	Day 29	0/12 (0)	0/10 (0)	4/71 (5.6)	4/78 (5.1)	0/6 (0)	0/10 (0)						
	Day 43	5/12 (41.7)	7/10 (70)	57/71 (80.3)	47/78 (60.3)	0/6 (0)	0/9 (0)						
	Day 57	-	-	-	14/21 (66.7)	-	-						
	Day 85	-	-	-	20/21 ^a (95.2)	-	-						
	Day 180	-	-	1/21 (4.8)	0/11 (0)	-	-						
	Day 208	-	-	21/21 ^b (100)	10/11 ^b (90.9)	-	-						
RBD IgG													
Seroconversion ^a , n/N (%)													
	Day 29	0/12 (0)	0/10 (0)	9/158 (5.7)	6/159 (3.8)	0/17 (0)	0/16 (0)						
	Day 43	9/12 (75.0)	7/10 (70)	143/157 (91.1)	116/157 (73.9)	0/17 (0)	1/15 (6.7)						
	Day 57	-	-	-	17/21 (81.0)	-	-						
	Day 85	-	-	-	20/21 ^a (95.2)	-	-						
	Day 180	-	-	16/21 (76.2)	3/11 (27.3)	-	-						
	Day 208	-	-	21/21 ^b (100)	11/11 ^b (100)	-	-						

^a Defined as a four-fold increase in titers over baseline (i.e., rise from Day 1).

^a Seroconversion rate at Day 85 for study group with booster at Day 57.

^b Seroconversion rate at Day 208 for study group with booster at Day 180.

[#] HAV = Hepatitis A vaccine, Pneumo = pneumococcal vaccine.

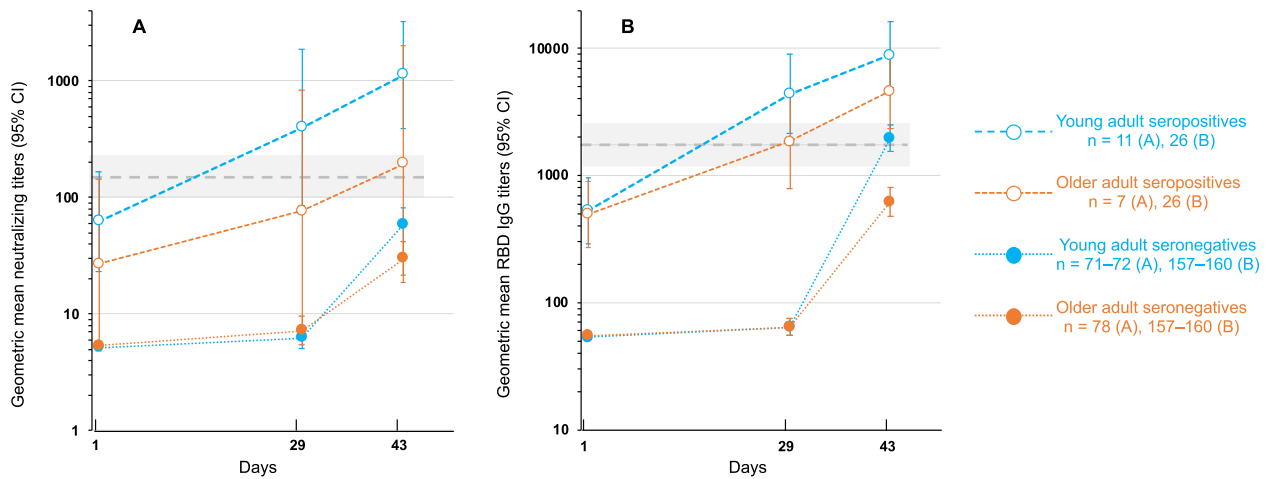


Fig. 5. Geometric mean titers (95% CI) of neutralizing antibodies (panel A) and receptor binding domain (RBD) IgG antibodies (panel B) following vaccination with 12 µg CVnCoV on Days 1 and 29 in younger and older adults according to their serostatus for SARS-CoV-2 at baseline (seronegative data as in Fig. 4). Lowest value considered as positive is 10 for neutralizing titers and 100 for RBD IgG titers. Grey dashed lines show the respective GMTs of 68 human convalescent sera (HCS), shading shows 95% confidence interval. Lowest value considered as positive is 10 for neutralizing titers and 100 for RBD IgG titers.

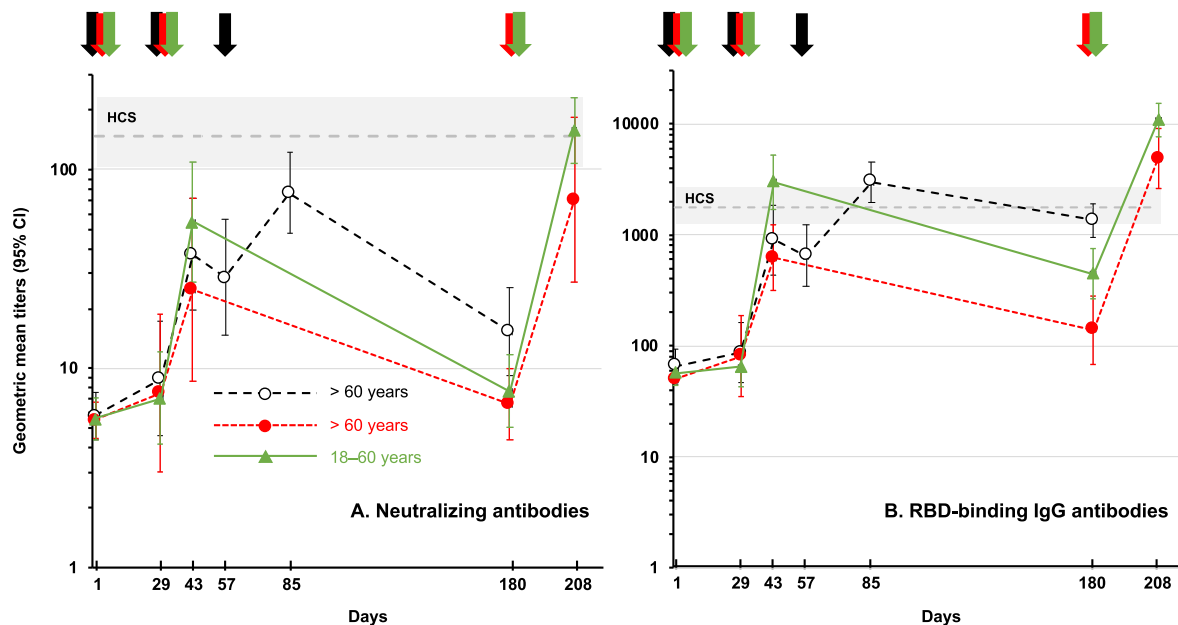


Fig. 6. Geometric mean titers (95% CI) of neutralizing (A) and receptor binding domain (RBD) (B) antibodies in the sub-set of older adults (>60 years) who received three 12 µg doses of CVnCoV on Days 1, 29 and 57 (n = 21, black line) as indicated by the black arrows, the subsets of younger (n = 21, green line) and older (n = 11, red line) adults who received three 12 µg doses of CVnCoV on Days 1, 29 and 180 (shown by red and green arrows). Grey dashed lines show the respective GMTs of the panel of 68 human convalescent sera (HCS), shading shows the 95% confidence interval. Lowest value considered as positive is 10 for neutralizing titers and 100 for RBD IgG titers.

binding IgG GMTs had waned further, to almost baseline levels for neutralizing antibodies. On Day 208, four weeks after the booster, 90.9% and 100% of individuals seroconverted for neutralizing and RBD IgG antibodies with GMFR of 10.6 and 34.7 and GMTs overlapping (neutralizing) or higher (RBD) than those observed in the HCS panel (Fig. 6). The same patterns of response were observed for the younger adults who received the Day 180 booster, with waning of antibodies to Day 180. Neutralizing and RBD-binding IgG were equal to or surpassed HCS levels, respectively (Fig. 6). The seroconversion rate was 100% for both neutralizing and RBD-binding antibodies with GMFR of 20.5 and 24.2, respectively.

Cellular immunogenicity

A total of 55 participants (35 18–60 year-olds and 20 > 60 year-olds) provided PBMCs for analysis on Days 1, 29 and 43. S-protein specific poly-functional CD4 + T cell responses were detectable in both age groups after the first dose. Responses were more robust by Day 43, two weeks after the second dose, illustrated by the increase in median frequencies (Fig. 7). Statistically significant (p < 0.05) CD4 + T cell responses were observed at Days 29 and 43 in both age groups for Sp1, Sp2, and Sp1 + Sp2 peptide pools (Supplementary table 4). No S-protein-specific CD4 + T cell response

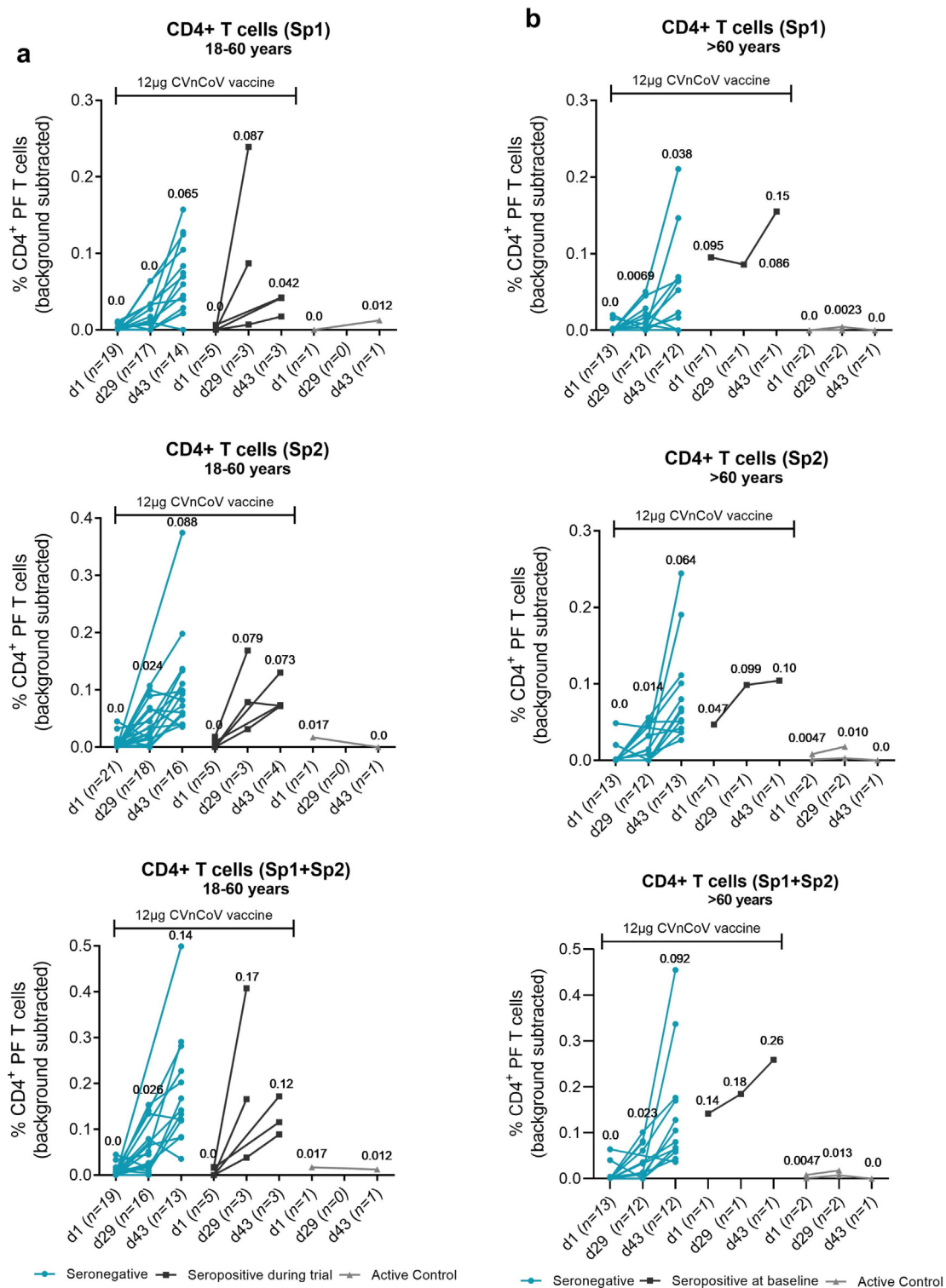


Fig. 7. SARS-CoV-2-Spike-specific CD4 + T cells measured by intracellular cytokine staining after stimulation with overlapping peptide pools (Sp1 and Sp2) covering the entire SARS-CoV-2-Spike protein. Sp1 and Sp2 induced T cell frequencies were background-subtracted and shown separately or summed to a total frequency of poly-functional (PF) CD4 + T cells expressing at least two markers among IFN- γ , TNF- α , IL-2 and CD40L in 18–60-year-olds (left panels) and > 60-year-olds (right panels). Total PF T cell frequencies were plotted before (Day 1) and after the first vaccination (Day 29), and after the second vaccination (Day 43) with a 12 μ g CVnCoV dose or an active control. All data from evaluable 18–60-year-olds who were either seronegative or seropositive during the trial are shown. Values indicate median of PF CD4 + T cells frequencies per time point.

was observed in any control vaccine recipient. Moreover, in seronegative participants, the CVnCoV-induced CD4 + T cell response on Day 43 was comparable to the responses detected in five 18–60 year-old participants who were exposed to natural

infection during the trial and one > 60 year-old participant who was seropositive at baseline (Fig. 7).

Low frequencies of SARS-CoV-2 S-protein specific CD8 + T cells were detected in both age groups compared with CD4 + T cells

(Supplementary Fig. 1). Low frequencies of SARS-CoV-2 S-protein specific CD8 + T cells were detected in both age groups compared with CD4 + T cells (Supplementary Fig. 1). In comparison with CD4 + T cells, antigen-specific CD8 + T cell responses were less consistent in both age groups.

Discussion

The phase 2a study was performed to confirm the optimal dose of 12 µg CVnCoV suggested by our phase 1 study of 18–60 year-olds, and to ensure that this dose would be safe and immunogenic in adults aged over 60 years. We have confirmed that immunogenicity of the 12 µg dose is higher than with the 6 µg dose in younger adults. Responses in older adults were lower than in younger participants with both 6 µg and 12 µg doses. The higher dose is more reactogenic, expressed mainly as short-lived mild to moderate solicited AEs which resolve within 3 days of vaccination. There were no vaccine-related SAEs or deaths, and no events raised DSMB concerns about safety in either age group. The reactogenicity profile of CVnCoV is similar to published reports of current COVID-19 vaccines [4,5]. As approximately 18–33% of SARS-CoV-2 infections are believed to be asymptomatic, it is important to note that the vaccine is safe in those with prior infection [17–19].

As we previously observed in our phase 1 study [7], we found that two doses of CVnCoV are necessary to induce a strong immune response in those who are immunologically naïve for SARS-CoV-2. In those with evidence of prior exposure to SARS-CoV-2, a marked immune response was apparent after one dose, and this response was further increased by the second dose. In older adults, the administration of a booster dose of CVnCoV at Day 57 resulted in a further incremental response with 95% seroconverting for both neutralizing and RBD-binding antibodies when measured four weeks later. Although titers of RBD-binding IgG antibodies persisted above baseline at Day 180, five months after the second vaccination, levels of neutralizing antibodies had waned to almost baseline levels at this timepoint. Homologous booster doses administered at Day 180 then resulted in 91% and 100% of older adults seroconverting for neutralizing and RBD-binding antibodies, and 100% of younger adults seroconverted for both. The response after the Day 180 booster was high in both immune assays for both age groups, and achieved GMTs with overlapping confidence intervals or higher than those observed in the HCS panel. It is notable that although 31 of 32 subjects did not show seroconversion for neutralizing antibodies on Day 180, almost all had seroconverted with high antibody levels after a booster dose. This confirms the existence of immunological memory in CVnCoV vaccinees induced by two doses, even in the absence of detectable antibodies in blood at the time point of boost vaccination.

S-protein-specific CD4 + T cell responses to two peptide pools, Sp1 and Sp2, which together cover the entire S-protein molecule, were induced by the first 12 µg dose in both age groups and enhanced by the second. The CD4 + Th1 T cell frequencies are consistent with those in persons who are seropositive due to previous infection at baseline, or who became seropositive due to infection during the trial as evidenced by N protein serology. Observation of absent or weak CD8 + T cell responses in younger or older adults measured by *ex vivo* ICS using bulk PBMCs is also consistent with previous observations after natural infection with SARS-CoV-2, as CD8 + T cells responses were found less consistently than CD4 + T cell responses in human convalescent patients [20]. This is probably due to the fact that 90% of the SARS-CoV-2 CD8 + T cell epitopes are found in other proteins [21].

In conclusion, the interim results of this study confirm the selection of 12 µg CVnCoV as the dose for further clinical development. This dose balances an acceptable reactogenicity profile with

humoral and cellular immune responses in young adults. In the older adult age group studied, there was some evidence of lower immune responses, likely due to immunosenescence, which could be overcome by a booster dose. Responses to two doses in both age groups were lower than those observed in convalescing patients after symptomatic COVID-19, but achieved those levels after homologous booster doses. While it is reassuring to observe robust immune responses, particularly after booster vaccinations indicating immune memory has been induced by two primary vaccinations in young and older adults, there is currently no validated serologic correlate of protection for SARS-CoV-2 vaccines against COVID-19. Only a clinical efficacy study of this vaccine candidate could confirm its effectiveness in preventing COVID-19. When the 12 µg dose was used in the phase 2b/3 efficacy study (HERALD) with nearly 40,000 adult participants to assess the efficacy against COVID-19 (EudraCT Number: 2020-003998-22; [ClinicalTrials.gov: NCT04652102](https://clinicaltrials.gov/ct2/show/study/NCT04652102)) the overall vaccine efficacy against symptomatic disease was 48.2% (95% CI: 31.0–61.4) [11]. The combination of low responses to the first two vaccinations and the rapid decline in antibodies may have left participants susceptible to infection before receiving the booster dose, consistent with the moderate efficacy observed in the HERALD study following the two-dose vaccination schedule [11].

In view of the overall efficacy and emergence of SARS-CoV-2 variants, the decision had been made to cease development of the CVnCoV candidate, to allow focus of further investigations on clinical studies of the second generation vaccine candidate, CV2CoV. The CV2CoV candidate has already demonstrated superior immunogenicity, with more rapid onset of higher humoral and cellular immune responses in non-human primate studies [22].

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The authors report article publishing charges, and writing assistance were provided by CureVac AG. XS-L, CRC, RC, LE, AIG, GL-R received institutional funding for the work; GL-R received consulting fees; HJ, SDK, SL, GQ, BS and O-OW are employees of the sponsor; MG, S-KK, PM, DV, PvE-R and LO are employees of the sponsor with stock options. Other authors declare no conflicts.

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Appendix A. Supplementary material

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