Clinical Research

Immunogenicity and safety of adenovirus-based vector vaccines for COVID-19: a systematic review and meta-analysis

Ayers Gilberth Ivano Kalaij,[1] Valerie Josephine Dirjayanto,[1] Syarif Maulana Yusuf,[2] Erni Juwita Nelwan[3]

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Authors' affiliations:
[1]Faculty of Medicine, Universitas Indonesia Jakarta, Indonesia, [2]Infectious Disease and Immunology Research Center, Indonesia Medical and Education Research Institute (IMERI), Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia, [3]Department of Internal Medicine, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

Corresponding author:
Erni Juwita Nelwan
Division of Tropical and Infectious Diseases, Department of Internal Medicine, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, Jalan Diponegoro No. 71, Kenari, Senen, Central Jakarta 10430, DKI Jakarta, Indonesia
Tel/Fax: +62-21-3914190
E-mail: erni.juwita@ui.ac.id

ABSTRACT

BACKGROUND Despite various research on vaccine development, severe acute respiratory syndrome coronavirus 2 infection continues to spread. Thus, developing a more effective vaccine for production and clinical efficacy is still in high demand. This review aimed to assess the immunogenicity and safety of adenovirus-based vector vaccines (Ad-vaccines) including Ad5-vectored, ChAdOx1 nCoV-19, rAd26-S or rAd5-S, and Ad26.COV2.S as the promising solutions for COVID-19.

METHODS We conducted a systematic review and meta-analysis of clinical trials based on the preferred reporting items for systematic reviews and meta-analyses guidelines through PubMed, Scopus, Cochrane, and EBSCOhost until August 17, 2021. We implemented inclusion and exclusion criteria and assessed the studies using the US National Toxicology Program's Office of Health Assessment and Translation risk of bias rating tool for human and animal studies. Pooled estimates of odds ratio (OR) were analyzed using fixed-effect model.

RESULTS This systematic review yielded 12 clinical studies with a total of 75,105 subjects. Although the studies were heterogeneous, this meta-analysis showed that Ad-vaccine significantly increased protection and immune response against COVID-19 with a pooled efficacy of 84.68% compared to placebo ($p<0.00001$). Forest plot also indicated that Ad-vaccine conferred protection against moderate to severe COVID-19 with a pooled OR of 0.26 ($p<0.00001$). Ad-vaccine had also shown a good safety profile with local site pain and fever as the most common side effects.

CONCLUSIONS Ad-vaccine had shown a good immunogenicity for COVID-19 with a good pooled efficacy and was proven safe for COVID-19 patients.

KEYWORDS adenovirus vaccine, COVID-19, immunogenicity, safety, SARS-CoV-2

Various types of vaccines have been studied worldwide. However, the limited efficacy and distribution difficulties of each vaccine have led to the inequality of access, especially in areas with limited resources. For instance, RNA-based vaccines require a temperature of –70°C during transportation, and the safety remains unknown since the technology is relatively new.[1] Meanwhile, inactivated virus-based vaccines are proven to be less effective in inducing the mucosal immune response.[2] Cost becomes a major concern for attenuated vaccines due to the expansive-scale inoculation programs that require millions of doses. Dendritic cell vaccine serves as another potential immune inducer due to the possible role of C-type lectin on the surface of dendritic cells in the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) invasion.[3] Currently, dendritic cell vaccine is also being studied for cancer prevention,

but the research of its application for coronavirus disease 2019 (COVID-19) is still limited. Therefore, studies for more effective vaccines are still needed today.

Adenovirus-based vector vaccine (Ad-vaccine) has been studied in several countries and is potentially capable to deliver specific antigen and induce innate and adaptive immune systems.[4] The Ad-vaccine has been developed to prevent other infections such as Ebola, HIV, tuberculosis, and malaria; hence, due to former experience, large-scale manufacture is more achievable.[5] In addition, some studies have established a method of freeze-drying the Ad-vaccines, enabling vaccine transport without any extremely cold temperature containers.[6] Thus, the COVID-19 Ad-vaccine provides prospective benefits that are relatively safe with more efficient manufacturing and distribution.

To the best of our knowledge, no systematic review of clinical studies analyzing the development of Ad-vaccine to prevent COVID-19 spread is currently available. This review aimed to identify the efficacy and safety of Ad-vaccine for SARS-CoV-2.

METHODS

We conducted a systematic review based on the preferred reporting items for systematic reviews and meta-analyses checklist (http://www.prisma-statement.org/).[7] The study protocol was registered on the International Prospective Register of Systematic Reviews (CRD42021233411).

Information sources and search strategy

We conducted a thorough literature search through multiple electronic databases, such as PubMed, Scopus, Cochrane, and EBSCOhost. The search included all studies published until August 17, 2021. The keywords used were "COVID-19", "Adenovir*", and "Vaccine". The unpublished clinical trials were searched in ClinicalTrials.gov. The literature searching was limited to clinical trial studies,

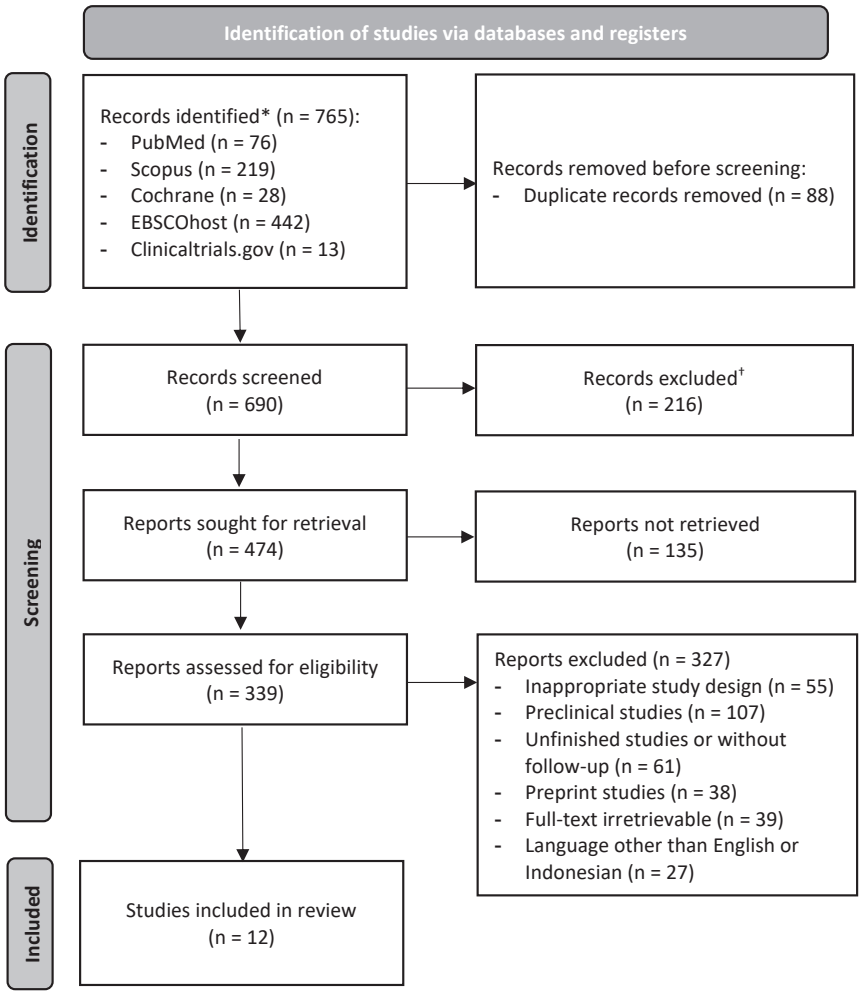

Figure 1. Flow diagram of the literature search strategy. *Databases literature searching using keywords described in the search strategy section; †records excluded due to irrelevant title and abstract

full-text availability, and studies written in English or Indonesian because they were the only languages compatible with the authors.

Study eligibility criteria

We further screened studies according to the following inclusion and exclusion criteria. Our inclusion criteria included (1) randomized and non-randomized clinical trials, (2) healthy subjects at any ages without a history of COVID-19 to adjust for the confounding factors, (3) Ad-vaccine as their intervention, (4) a placebo utilization or not given any vaccines as their control, and (5) efficacy (in terms of immunogenicity) and safety identification of Ad-vaccine toward COVID-19 as the study outcome. Our exclusion criteria included (1) preprint studies, (2) unfinished studies, (3) studies without follow-ups, (4) full-text irretrievable studies, and (5) studies written in languages other than English or Indonesian.

Study selection

Duplicated studies were removed using EndNote X9 software (Clarivate Analytics, USA). Two independent reviewers (AGIK and VJD) screened the titles and abstracts according to the accessibility criteria. The literature search is shown in Figure 1.

Data extraction

Data of the included studies were extracted by two independent reviewers (AGIK and VJD). These data included: author and publication year, study location, study design including phase and blinding, subject characteristics, intervention, follow-up duration, and outcomes for the efficacy and safety of Ad-vaccines.

Data synthesis

Quantitative analysis was performed using Review Manager 5.4 (The Nordic Cochrane Center, The Cochrane Collaboration, Denmark) with an inverse variance and fixed-effect model. Odds ratios

Table 1. OHAT risk of bias tool for animal and human studies

First author, year	Selection bias		Performance bias		Attrition/ exclusion bias	Detection bias		Selective reporting bias	Other bias
	Randomization	Allocation concealment	Identical experimental conditions	Blinding during study	Incomplete outcome data	Confidence in exposure characterization	Confidence in outcome assessment	Outcome reporting	Other potential threat to internal validity
Zhu,[9] 2020	++	++	+	++	++	NR	++	++	++
Zhu,[10] 2020	-	+	++	NR	+	NR	+	+	NR
Folegatti,[11] 2020	++	++	++	++	++	+	++	++	++
Logunov,[12] 2020	-	+	++	-	++	+	+	++	NR
Stephenson,[13] 2021	++	++	++	++	+	++	++	++	+
Sadoff,[14] 2021	++	++	+	++	+	++	+	+	+
Sadoff,[15] 2021	++	++	+	++	+	++	++	++	+
Ramasamy,[16] 2020	++	+	++	+	++	++	++	++	+
Madhi,[17] 2021	++	++	++	++	+	+	++	++	+
Logunov,[18] 2021	++	++	+	++	++	++	+	++	+
Ewer,[19] 2020	++	++	++	++	+	+	++	++	+
Emary,[20] 2021	++	+	++	+	+	++	++	+	NR

OHAT=the US National Toxicology Program's Office of Health Assessment and Translation
+=probably low risk of bias; ++=definitely low risk bias; NR=probably high risk of bias

(ORs) and 95% confidence intervals were extracted, and any missing data found would be completed by contacting the corresponding author of the study. Statistical heterogeneity was evaluated by Cochran's Q test and I^2 statistics, with the cut-off values of 0%, 25%, 50%, and 75% for insignificant, low, moderate, or high heterogeneity, respectively. An OR of <1 showed good immunogenicity for Ad-vaccine compared to placebo. The pooled efficacy was also assessed using forest plot of rate ratios to estimate the efficacy of Ad-vaccine among studies. Furthermore, the sensitivity was analyzed using Duval and Tweedie's trim-and-fill analysis due to the potential substantial heterogeneity.[8]

Outcome assessment was further done and discussed. The immunogenicity and safety were assessed quantitatively and then interpreted into narrative analysis if the data were too heterogeneous. Immunogenicity was measured using the data of seroprotection rate, seroconversion rate, and increase or response titers of neutralizing or enzyme-linked immunosorbent assay (ELISA) antibodies against SARS-CoV-2 virus and its proteins up to 28 or 56 days, depending on the study. The safety was evaluated by the authors' reports on solicited and unsolicited adverse events, systemic adverse events, adverse reactions, and safety profile of Ad-vaccine as compared to the control injections; all data were combined qualitatively into a summary of the study table. Furthermore, a forest plot estimating the average of antibodies count to the receptor-binding domain (RBD) at day-0 and -28 after the administration of the COVID-19 vaccine was also used to further summarize the efficacy of the vaccine.

Risk of bias and quality assessment

Quality assessment of the studies was performed using the US National Toxicology Program's Office of Health Assessment and Translation (OHAT) risk of bias rating tool for human and animal studies.[6] This tool assesses selection bias, performance bias, exclusion bias, detection bias, selective reporting bias, and other biases. A good quality study should have at least four out of six aspects indicating low risks of bias. Quality assessment was done by two reviewers (AGIK and VJD) collaboratively, and discrepancies were consulted and resolved by the third reviewer (EJN) until consensus was reached. The risk of bias assessment is provided in Table 1.

RESULTS

The initial search from Pubmed, Scopus, Cochrane, and EBSCOhost resulted in 765 studies. After the title and abstract screenings, as demonstrated in Figure 1 (n = 339), 327 studies were further excluded because 55 had unsuitable study design, 107 were preclinical studies, 61 were unfinished or without follow-up, 38 were preprint studies, 39 had irretrievable full-text, and 27 were written in languages other than English or Indonesian. The search yielded 12 clinical studies, which were further included in the qualitative and quantitative synthesis.

Study characteristics and design

The detailed data extraction and characteristics of the included studies are shown in Table 2. Overall, this review included a total of 75,105 subjects with study locations varied worldwide: two studies were conducted in China, four studies in the UK, one study in South Africa, two studies in the USA, one study in the Netherlands, and two studies in Russia. Almost all clinical trials applied similar study designs, which can be compared reliably. Outcomes were the efficacy (in terms of immunogenicity) and safety of Ad-vaccine.[9–20]

Quality of studies

Studies were also assessed for their quality based on the OHAT risk of bias rating tool for human and animal studies, as shown in Table 1. Randomization bias was unclear in this review (two studies did not report the method), followed by detection bias and other biases (one study for each bias). However, most studies had good quality, indicating that this review included relatively good studies.

Qualitative analysis of safety and efficacy

The qualitative analysis of the studies is summarized in Table 2. All studies demonstrated good efficacy of Ad-vaccines for inducing an immune response. In general, the adaptive immune response had reached substantial levels from day-14 to -28, particularly the specific anti-spike immunoglobulin G (IgG) chimpanzee adenovirus-vectored vaccine (ChAdOx1 nCoV-19), RBD (recombinant adenovirus type 26 carrying the gene for SARS-CoV-2 full-length glycoprotein S [rAd26-S], recombinant adenovirus type 25 carrying the gene for SARS-CoV-2 full-length glycoprotein S [rAd5-S]), neutralizing

Table 2. Summary of study characteristics

First author, year	Study location	Study design		Subject characteristics	Intervention	Follow-up duration	Outcomes	
		Study phase	Blinding				Efficacy (immunogenicity)	Safety
Zhu,[9] 2020	Wuhan, China	II	Double-blind	508 people aged 18 years and above, had no history of HIV and SARS-CoV-2 virus infection Mean (SD) age: 39.7 (12.5) years old	IM injection of a single dose of Ad5-vectored COVID-19; dosage: (I) 1×10^{11} viral particles/ml, (II) 5×10^{10} viral particles/ml, or placebo.	Immunogenicity = 28 days Safety = 14 days	ELISA antibody specific to RBD was formed. Seroconversion rates were 96% and 97%. Ad-vaccine induced neutralization antibody response significantly toward the living virus. IFN-ɣ ELISpot response post-vaccination with seroprotection rate of 90% and 88%.	72% (I) and 74% (II) of respondents experienced solicited adverse events. 9% (I) and 1% (II) of respondents experienced severe adverse events. No serious adverse events
Zhu,[10] 2020	Wuhan, China	I	NR	108 healthy people aged 18–60 years	IM injection of a single dose of Ad5-vectored COVID-19; dosage: 5×10^{10}, 1×10^{11}, and 1.5×10^{11} viral particle/ml	Immunogenicity = 14 and 28 days Safety = 7 days (adverse events) and 28 days post-vaccination	Significant increased of ELISA and neutralization antibody 14 days post-vaccination. Neutralization antibody titer increased 4 times fold in most respondents (5%). T-cell specific response peaked at 14 days post-vaccination.	All dosages reported solicited adverse events. The most common adverse events reported was pain (54%). Systemic adverse events reported were fever (46%) and fatigue (44%).
Folegatti,[11] 2020	Oxford, UK	I/II	Single-blind, RCT	1,077 healthy people aged 18–55 years without any history of SARS-CoV-2 lab-confirmed infection or having COVID-19-related symptoms	IM injection: ChAdOx1 nCoV-19 with the dosage: 5×10^{10} viral particles (n = 543) MenACWY (1 dose IM) ChAdOx1 nCoV-19 prime-boost group after 28 days	Immunogenicity = 56 days Safety = 7 days	91% of respondents reported to have antibodies toward SARS-CoV-2 in MNA_{80} and 100% in $PRNT_{50}$. Booster increased the host antibodies toward SARS-CoV-2. Neutralizing antibody response, T-cell specific response toward spike, and IgG anti-spike increased significantly ($p<0.001$).	Pain, fever-like, shivering, muscle spasm, headache, and malaise ($p<0.05$) were reported as adverse events post-vaccination; however, these adverse events resolved with prophylaxis paracetamol. No serious adverse events

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Table 2. (continued)

First author, year	Study location	Study design		Subject characteristics	Intervention	Follow-up duration	Outcomes	
		Study phase	Blinding				Efficacy (immunogenicity)	Safety
Logunov,[12] 2020	Moscow, Russia	I/II	Non-blinded, non-RCT	76 healthy people aged 18–60 years (38 in two study phases)	Phase I: IM injection of a single dose of rAd26-S or rAd5-S Phase II: 5 days post-phase I, prime-boost rAd26-S at day-0, and rAd5-S at day-21	28 days	All participants produced antibody toward SARS-CoV-2 glycoprotein. At day-42, RBD specific IgG titer was increased significantly (p<0.05). Neutralizing antibodies produced were 49.25. Seroconversion rate was 100%. CD4+ and CD8+ cell responses were detected in 100% of participants at day-28.	Both vaccines were safe and well tolerated. Common adverse events reported: injection reported were injection site effects (58%), hyperthermia (50%), and headache (42%). No serious adverse events
Stephenson,[13] 2021	Boston, USA	I/IIa	Multicenter, double-blind, placebo-controlled trial	25 adults aged 18–55 years and negative for SARS-CoV-2 infection by nasopharyngeal PCR and serum Ig testing	IM injection of Ad26.COV2.S at 5×10^{10} or 1×10^{11} viral particles for single-shot or two-shot vaccine schedules, 56 days apart	71 days	90% of participants experienced a rapid increase of binding antibodies by 8 days. 25% of participants experienced a rapid increase of neutralizing antibodies by 8 days. Binding and neutralizing antibodies were detected in 100% of participants after a single-shot. GMT of spike-specific binding antibodies were 2,432 to 5,729, and neutralizing antibodies were 242 to 449 after 71 days. CD4+ and CD8+ T-cell responses were induced in all but one participant. Varied antibody subclasses, Fc receptor binding properties, and antiviral functions were induced.	-

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Table 2. (continued)

First author, year	Study location	Study design		Subject characteristics	Intervention	Follow-up duration	Outcomes	
		Study phase	Blinding				Efficacy (immunogenicity)	Safety
Sadoff,[14] 2021	Leiden, Netherlands	III	International, randomized, double-blind, placebo-controlled trial	The per-protocol population included 19,630 SARS-CoV-2-negative participants who received Ad26.COV2.S and 19,691 who received placebo.	IM injection of a single dose of Ad26.COV2.S 5×10^{10} viral particles	58 days	After 14 days, Ad26.COV2.S had protection against moderate to severe/critical COVID-19 with a 66.9% efficacy (adjusted 95% CI: 59.0–73.4); 116 cases in the vaccine group vs. 348 in the placebo group. After 28 days, the efficacy was 66.1% (adjusted 95% CI: 55.0–74.8); 66 vs. 193 cases. Efficacy of vaccine was 52.0% after 14 days and 64.0% after 28 days against severe-critical COVID-19 caused by 20H/501Y.V2. Less number of deaths in the vaccine group (3, none COVID-related) than the placebo (16, 5 COVID-related)	Injection site pain in 48.6% of participants, systemic reactions including headache (38.9%), fatigue (38.2%), myalgia (33.2%), and nausea (14.2%) Venous thromboembolic events were commonly occurred in vaccine (11) than in placebo (3). Seizures were commonly occurred in the vaccine group (4) compared to the placebo (1). Tinnitus was commonly occurred in the vaccine group (6) compared to the placebo (0).

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Table 2. (continued)

First author, year	Study location	Study design		Subject characteristics	Intervention	Follow-up duration	Outcomes	
		Study phase	Blinding				Efficacy (immunogenicity)	Safety
Sadoff,[15] 2021	Belgium and USA	I-IIa	Multicenter, placebo-controlled trial	Healthy adults aged between 18–55 (cohort 1, n = 402) and 65 years or older (cohort 3, n = 403)	IM injection of Ad26.COV2.S vaccine at 5×10^{10} viral particles (low dose) or 1×10^{11} viral particles (high dose) per milliliter or placebo in a single-dose or two-dose schedule	71 days	Cohort 1a At day-29 after first vaccine, neutralizing antibody was detected in 90% or more of participants (GMT: 224 to 354). At day-57 after the first vaccine, neutralizing antibody was detected in 100% of participants (GMT: 288 to 488). Titers remained stable until day-71. Second dose increased titer by a factor of 2.6–2.9 (GMT: 827 to 1,266). Spike-binding antibody response was similar to neutralizing antibody response. At day-14 CD4+ T-cell response was detected in 76–83% of participants. Cohort 3 At day-14 CD4+ T-cell response was detected in 60–67% of participants. CD8+ response was robust but lower than in thecohort 1.	Cohort 1 Most common reactions included fatigue, headache, myalgia, and pain in the injection site. Most frequent systemic adverse event included fever. Low-dose experienced less adverse effects. Cohort 3 Systemic adverse events were less common than in the cohort 1. Low-dose experienced less adverse effects.

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Table 2. (continued)

First author, year	Study location	Study design		Subject characteristics	Intervention	Follow-up duration	Outcomes	
		Study phase	Blinding				Efficacy (immunogenicity)	Safety
Ramasamy,[16] 2020	Oxford, UK	II/III	Single-blind, RCT	160 participants aged 18–55 years, 160 aged 56–69 years, and 240 aged 70 years and older with no uncontrolled comorbidities or high frailty score	IM injection of ChAdOx1 nCoV-19 (2.2×10^{10} viral particles) or a control vaccine, MenACWY Prime-booster regimens were given 28 days apart. Then, participants were given the standard-dose ($3.5–6.5 \times 10^{10}$ viral particles of ChAdOx1 nCoV-19).	56 days	Two vaccine doses Median anti-spike SARS-CoV-2 IgG responses 28 days after the boost dose were similar across the three age cohorts (standard-dose groups: 18–55 years, 20,713 AU/ml [IQR: 13,898–33,550], n = 39; 56–69 years, 16,170 AU/ml [IQR: 10,233–40,353], n = 26; and ≥70 years 17,561 AU/ml [IQR: 9,705–37,796], n = 47; $p = 0.68$). Neutralizing antibody titers after a boost dose were similar across all age groups (median MNA_{80} at day-42 in the standard-dose groups: 18–55 years, 193 [IQR: 113–238], n = 39; 56–69 years, 144 [IQR: 119–347], n = 20; and ≥70 years, 161 [IQR: 73–323], n = 47; p = 0.40). 14 days after the booster, 208 (>99%) of the 209 boosted participants had neutralizing antibody responses. 14 days after single standard dose, T-cell responses were peaked (18–55 years: median 1,187 SFC per million PBMCs [IQR: 841–2,428], n = 24; 56–69 years: 797 SFC [IQR: 383–1,817], n = 29; and ≥70 years: 977 SFC [IQR: 458–1,914], n = 48).	Local (injection-site pain, feeling feverish) and systemic reactions (muscle ache, headache) were more common in participants given ChAdOx1 nCoV-19 than in the control. Reactions were less common in adults aged 56 years or more. Local reactions were reported in 43 (88%) of 49 participants in the 18–55 years old group, 22 (73%) of 30 in the 56–69 years old group, and 30 (61%) of 49 in the 70 years old and more group. Systemic reactions were found in 42 (86%) participants in the 18–55 years old group, 23 (77%) in the 56–69 years old group, and 23 (65%) in the 70 years old and more group.
Madhi,[17] 2021	South Africa	IB-II	Double-blind, randomized, placebo-controlled multisite trial	2,026 HIV-negative adults (1,013 placebo and 1,013 vaccine participants) Age range: 18–65 years old; median age: 30 years old	IM injection of 0.33-to-0.5 ml dose of ChAdOx1 nCOV-19 vaccine or placebo with 21–35 days apart for the second dose.	Efficacy: 14 days after the second injection Solicited local and systemic reactogenicity: 7 days Unsolicited adverse events: 28 days	Humoral strong neutralizing antibodies 28 days after the first dose (GMT: 132 [IQR: 20–404]); rose further after second dose (GMT: 277 [IQR: 124–526]) against the B.1.351 variant. 21.9% of efficacy for mild-to-moderate COVID-19 due to the B.1.351 variant pseudovirus, and the live virus neutralization assays showed a greater resistance to the B.1.351 variant in the vaccine group.	Similar incidence of adverse events and serious adverse events between the vaccine and placebo recipients Serious adverse events: fever above 40°C after the first dose, resolved within 24 hours

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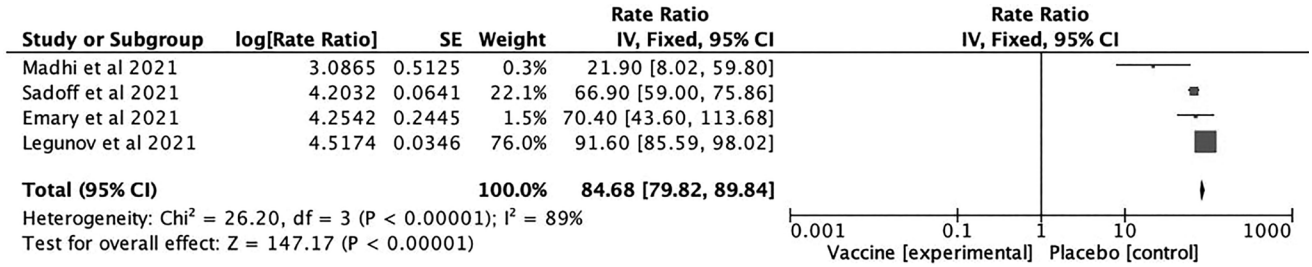
Table 2. (continued)

First author, year	Study location	Study design		Subject characteristics	Intervention	Follow-up duration	Outcomes	
		Study phase	Blinding				Efficacy (immunogenicity)	Safety
Logunov,[18] 2021	Moscow, Russia	III	Double-blind, placebo-controlled, RCT	21,977 adults (16,501 vaccine group and 5,476 placebo group). Mean (SD) age: 45.3 (12) years old	IM injection of 0.5 ml dose of rAd26 (first dose) and rAd5 (second dose) in a prime-boost regimen (21 days-interval between rAd26 and r5)	Efficacy: 21 days after the first dose Safety: day-28, -42, and -180	Neutralizing antibodies were detected at 42 days after the first dose (GMT: 44.5 [95% CI: 31.8–62.2]). 91.6% of efficacy for mild-to-moderate COVID-19 due to the B.1.351 variant 100% of efficacy from at least day-21 from the first dose against moderate or severe COVID-19 RBD-specific IgG was detected in 98% of samples (GMT: 8,996 [95% CI: 7,610–10,635]. Seroconversion rate was 98.25%.	Well tolerated Most reported adverse events were grade 1 (94.0% of 7,966 total events). No serious adverse events related to the vaccine
Ewer,[19] 2020	Oxford, UK	I/II	Single-blind, randomized multicentre controlled trial	88 participants aged 18–55 years	IM injection of ChAdOx1 nCov-19 at 5 x 10^{10} viral particles or control vaccine (MenACWY)	7, 14, 28, and 58 days blood samples evaluation for the efficacy and safety	Increased expression of CD69 in $CD4^+$ T cells at days 7-28; increased Ki-67 expression at day-7 and -14 after vaccination Anti-SARS-CoV-2 IgG responses were detected at day-14, peaked at day-28, and maintained at day-56. Increased levels of SARS-CoV-2 spike-specific IgM and IgA, peak responses at day-14 or -28	NR
Emary,[20] 2021	UK	II/III	Single-blind, multicenter, RCTs	8,534 adults aged 18 years and older, enrolled at 19 study sites in England, Wales, and Scotland	IM injection (1:1 ratio) receiving 5 x 10^{10} viral particles of ChAdOx1 nCoV-19 vaccine or MenACWY as a control with an interval of 14 or 28 days between the doses and booster doses	Weekly upper airway swab starting at day-14, -28, and more after the second dose of vaccine	Laboratory virus neutralization activity by vaccine-induced antibodies was lower against the B.1.1.7 variant compared to the Victoria lineage (GMT: 8.9 [95% CI: 7.2–11.0]). 70.4% of clinical efficacy against the symptomatic NAAT positive infection for the B.1.1.7 variant; 81.5% for the non-B.1.1.7 lineages Vaccine group had a shorter NAAT positive period (median: 1 week) compared to the control vaccine (median: 2 weeks). Live virus neutralizing titers of ChAdOx1 nCoV-19 recipient serum were nine times lower against the B.1.1.7 lineage than the Victoria lineage (GMT: 8.9 [95% CI: 7.2–11.0]).	NR

Ad=adenovirus; Ad26.CoV2.S=adenovirus serotype 26 vector expressing a stabilized SARS-CoV-2 spike; Ad5=adenovirus serotype 5; AU=arbitrary units; ChAdOx1 nCoV-19=chimpanzee adenovirus-vectored vaccine; CI=confidence interval; COVID-19=coronavirus disease 2019; ELISA=enzyme-linked immunosorbent assay; ELISpot=enzyme-linked immunospot; GMT=geometric mean titers; IFN=interferon; Ig=immunoglobulin; IM=intramuscular; IQR=interquartile range; MenACWY=meningococcal conjugate vaccine; MNA=microneutralization assay; NAAT=nucleic acid amplification test; NR=not reported; PBMC=peripheral blood mononuclear cell; PCR=polymerase chain reaction; PRNT=plaque reduction neutralization assay; rAd5-S=recombinant adenovirus type 5 carrying the gene for SARS-CoV-2 full-length glycoprotein S; rAd26-S=recombinant adenovirus type 26 carrying the gene for SARS-CoV-2 full-length glycoprotein S; RBD=receptor-binding domain; RCT=randomized controlled trial; SARS-CoV-2=severe acute respiratory syndrome coronavirus 2; SD=standard deviation; SFC=spot-forming cells

a. Forest plot analysis



b. Sensitivity analysis

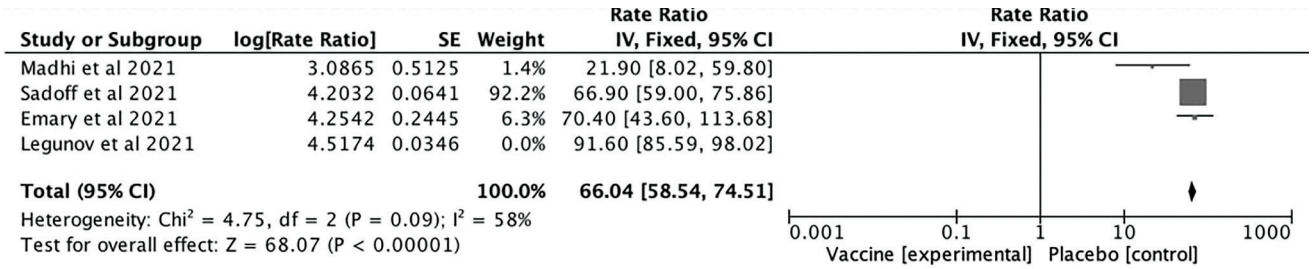
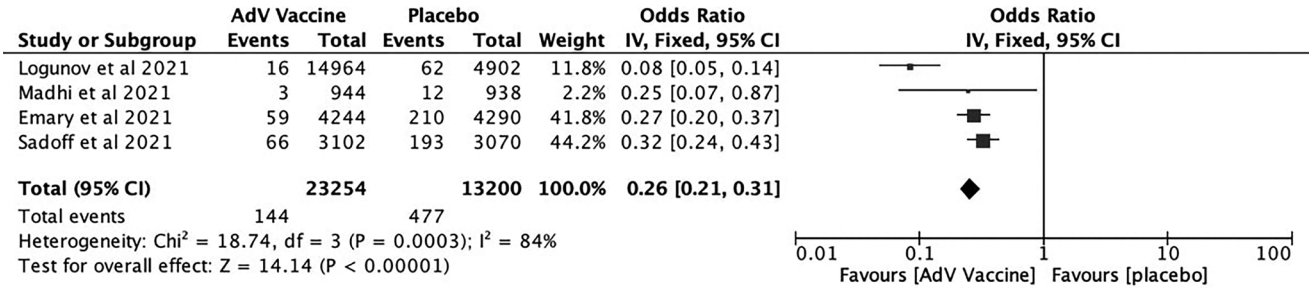


Figure 2. Analysis of the immunogenicity of Ad-vaccine against COVID-19. Ad-vaccine=adenovirus-based vector vaccine; COVID-19= coronavirus disease 2019

a. Quantitative analysis



b. Sensitivity analysis

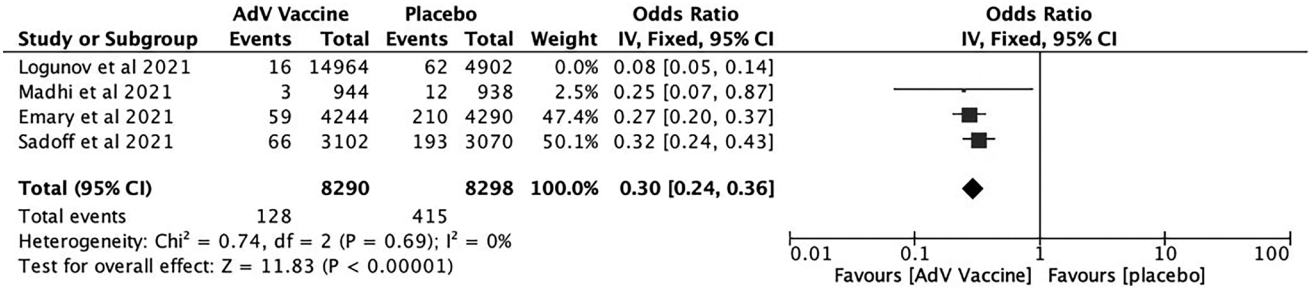


Figure 3. Analysis based on ORs of Ad-vaccine protection against moderate to severe COVID-19. Ad-vaccine=adenovirus-based vector vaccine; COVID-19= coronavirus disease 2019; OR=odds ratio

antibody (adenovirus serotype 26 vector expressing a stabilized SARS-CoV-2 spike [Ad26.COV2.S]), and $CD4^+$ or $CD8^+$ T-cell responses. Local adverse effects including injection site pain were found, and systemic adverse effects such as fever were also reported. However, only Sadoff et al[14] reported more severe side effects such as seizures and venous thromboembolic events.

Quantitative analysis of the efficacy of Ad-vaccine toward COVID-19 and sensitivity analysis

Our analysis confirmed that Ad-vaccine showed a pooled efficacy of 84.68% and was significant against the placebo (p<0.00001), as shown in Figure 2. Sensitivity analysis was performed using Duval and Tweedie's trim-and-fill analysis because the heterogeneity (I^2) was substantial (89%).[8]

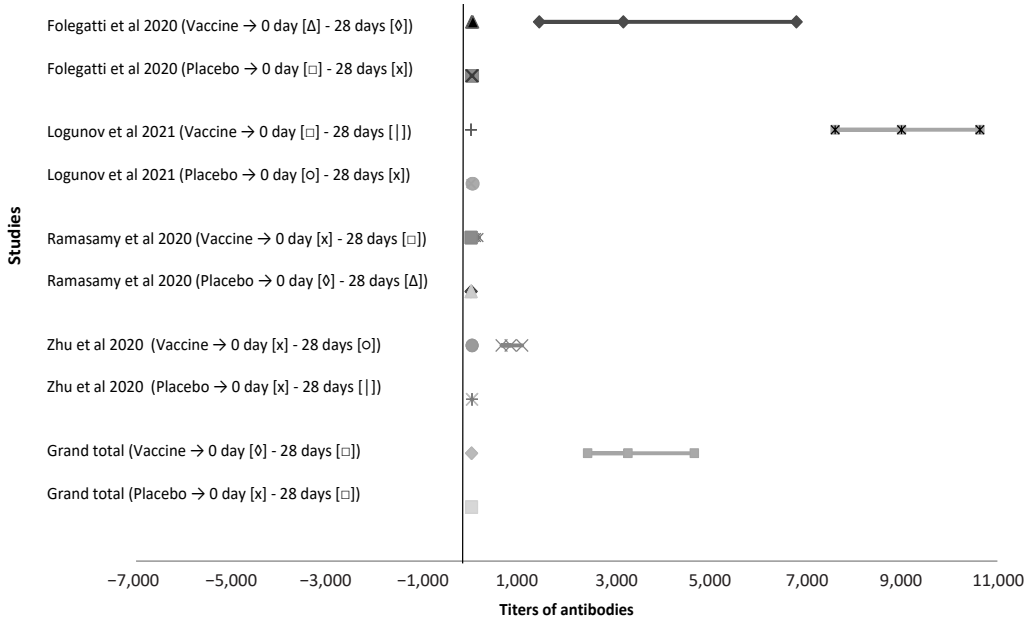


Figure 4. Forest plot estimating the average of antibodies count to RBD at day-0 and -28 after the COVID-19 vaccine administration. COVID-19=coronavirus disease 2019; RBD=receptor-binding domain

The exclusion of Logunov et al's[12] study in the sensitivity analysis resulted in a pooled efficacy of 66.04% (p<0.0001), with a low heterogeneity (I^2 = 58%).This might be due to a large number of participants included in this study, resulting in more variable outcome measures.[17]

Figure 3 shows the forest plot for the OR of Ad-vaccine protection against moderate to severe COVID-19. The pooled OR was 0.26, with the test for overall effect revealing significant results (p<0.00001). Similar to the previous analyses, substantial heterogeneity was found (I^2 = 84%) but dropped to I^2 = 0% based on Duval and Tweedie's trim-and-fill analysis[8] without Logunov et al's[12] study.

Antibody count

The average antibodies against RBD at day-0 and -28 after the COVID-19 vaccine administration were demonstrated by geometric mean titers (GMT). As shown in the Figure 4, the administration of the COVID-19 Ad-vaccine increased the RBD antibodies at day-28 in all studies. However, Zhu et al[9] did not demonstrate ELISA antibodies to RBD at day-0.

DISCUSSION

Based on the quantitative analysis, the pooled efficacy of Ad-vaccine was 84.68% and significant against placebo (p<0.00001), establishing its high effectivity in inducing antibody response. Previous preclinical studies in mammals also demonstrated the similar efficacy of COVID-19 Ad-vaccines in inducing sufficient immune response. In general, the Ad-vaccine elicited high antibody titers, especially IgG, against the S protein antigen. Wu et al,[21] who evaluated the intramuscular and intranasal administrations of adenovirus type 5-based COVID-19 vaccines (Ad5-nCoV) in BALB/c mice, found that even a single 5×10^8 low-dose vaccination provided a complete protection of the upper airways and lungs against infection. Each intervention at various doses elicited cellular, IgA, neutralizing antibodies, and S-specific IgG responses, with the intranasal group demonstrating higher IgG titers than the intramuscular group on week-6 and -8 (p = 0.0001).[19] Furthermore, Hassan et al[22] stated that chimpanzee Ad-vaccine (ChAd-SARS-CoV-2) resulted in significant anti-S IgG and T cell responses for intramuscular administration in BALB/c mice, reducing COVID-19 lung infection, as well as all anti-S IgG, IgA, and T cell responses for intranasal administration that annihilated lung infection. This was in line with previous studies which emphasized the role of good-quality CD4 and CD8 T-cells responses in protecting animals and humans against SARS-CoV-2 infection.[23] Another study in mice found the higher $CD8^+$ T cell responses, expressed through their elevated interferon gamma (IFNγ) and tumor necrosis factor-alpha (TNF-α).[24] This showed that a more effective cytotoxic T cell response was induced, in which its exhaustion was characterized

by NKG2 expression, resulting in progression to more severe disease.[25] Meanwhile, T helper 2 response against vaccine as shown by interleukin (IL)-4 and IL-10 expression was lower than the $CD8^+$ response.[24] Thus, this provided the basis for potential successful application in humans.

In congruence with the preclinical studies, the Ad-vaccine developed for clinical studies showed good results, including the Ad5-vectored, rAd26-S, rAd5-S, ChAdOx1, and Ad26.COV2.S vaccines. The Ad-5 vectored vaccine developed by Zhu et al[10] was a liquid preparation developed by cloning the spike gene and tissue plasminogen activator signal peptide gene and inserting them to the Ad5 with the deleted E1 and E3. The vaccine was then administered intramuscularly to the study subjects. Similarly, the recombinant adenovirus-vectored vaccines of rAd26-S and rAd5-S developed by Logunov et al[12] contained the genes for glycoprotein S in two different formulations, namely frozen and lyophilized vaccines. Folegatti et al[11] developed a chimpanzee adenovirus vector to express the spike protein through the ChAdOx1 formulation. Meanwhile, Ad26.COV2.S utilized adenovirus serotype 26 as a vector to produce the spike protein when the virus could not replicate.[13]

These Ad-vaccines showed good efficacy in inducing immune protection against COVID-19. Adaptive immune responses could be observed through the production of IgG against the spike protein or the RBD, neutralizing antibody responses to SARS-CoV-2, and T-cell responses. Post-vaccination seroconversion rates of all vaccines reached prominent results, particularly in Zhu et al's[10] study for binding antibodies, reaching an almost complete proportion of 97% and in Logunov et al's[12] study for antigen-specific IgG, which reached 100% at both day-28 and -42. Folegatti et al[11] found that anti-spike IgG also rose since the 28th day into a value of 157 ELISA units. In Stephenson et al's[13] study, a single vaccine shot induced binding and neutralizing antibody production in 100% of participants. Moreover, antibodies to the RBD were significantly elevated in all studies, which was an important finding because of its high affinity for the angiotensin-converting enzyme 2, the site of attachment, fusion, and access for the virus.[26] Antibodies prevent the virus from entering human cells, thus reducing the probability of infection.

For the new 20H/501Y.V2 COVID-19 variant, Sadoff et al[14] demonstrated that Ad26.COV2.S vaccine had a 64.0% efficacy against severe-critical disease, and Emary et al[20] demonstrated a 70.4% clinical efficacy for the ChAdOx1 formulation. This was crucial because those vaccines were less protective against the new variant. As a comparison, Madhi et al[17] found a low efficacy of 51% in the post-hoc NVX-CoV2373 nanoparticle vaccine against the same new variant. Meanwhile, the ChAdOx1 formulation had a 21.9% efficacy in preventing mild diseases,[17] which was also significantly lower. However, the crucial role of vaccines in preventing severe diseases was more prominent, which allowed a better patient prognosis.

The T-cell responses due to the vaccine were also found to be effective, as seen in the rise of IFN-γ from $CD4^+$ and $CD8^+$ T-cells, as well as TNF-α, and more prominently from $CD8^+$. The elevated $CD4^+$ response characterized by the higher IL-2 was also found, together with polyfunctional phenotypes from memory cells.[10] T-cell responses to COVID-19 rose in Logunov et al's[12] study, in which $CD4^+$ and $CD8^+$ had a median proliferation of 2.5% and 1.3% due to the frozen formulation and 1.3% and 1.1% due to the lyophilized formulation, respectively. Folegatti et al[11] found that T-cell responses specific for the spike antigen increased to their maximum value at day-14, with a median of 856 spot-forming cells per million peripheral blood mononuclear cells. After 7–28 days, $CD69^+$ $CD4^+$ T cell response in Ewer et al's[19] study had increased well, but Sadoff et al[15] showed a better CD4+ response in the younger age groups (18–55 years, 76–83%) than in the older cohort (65 or older, 60–67%). Consistent with the animal preclinical studies, the elevated helper $CD4^+$ T-cells in humans were essential for the maturation of antibodies and the corresponding cytotoxic effect of T-cells.[27] This strong T-cell response was found in COVID-19 asymptomatic and mild patients; thus, the elevation effect due to Ad-vaccine conferred essential protection against disease progression.[28]

However, the pre-existing neutralizing antibody titer could dampen the seroconversion; thus, Ad-vaccines were more suitable for people without a history of virus exposure.[10] Older participants between 45–60 years of age included in Zhu et al's[10] study also showed a reduced neutralizing antibody response compared to the younger participants. Thus, this particular vaccine might be more suitable for those below 45 years of age. Evaluating the dose might resolve this problem because vaccination with 1.5×10^{11} viral particles resulted in elevated neutralizing

antibody titers and increased the proportion of individuals with a 4-fold increase in antibody, compared to the low (5×10^{10}) and medium (1×10^{11}) doses.[10] In addition, additional vaccine boosters, as in the preclinical trials, also showed superior effects in inducing immunity. Folegatti et al[11] discovered a 100% neutralizing activity at day-42 for MNA_{80} and day-56 for microneutralization assays due to the booster. Logunov et al[12] found that rAd5-S booster elevated IgG titers, specifically after 7 days, the GMT rose to 5,382 in the frozen formulation and 5,322 in the lyophilized formulation. The lyophilized formulation is also more practical as it can be stored at 2–8°C, compared with the frozen formulation that should be stored at -18°C.[12] Compared to prime-only vaccination, the rAd26-S vaccine also had a significant increase in GMT value of 1,866 for frozen formulation and 1,372 for the lyophilized formulation after boosters.[12]

The Ad-vaccine was well-tolerated with minimum adverse effects, as shown in the preclinical trials. Some subjects reported local adverse reactions, such as pain, swelling, itching, and muscle weakness, but it was mild and manageable. These reactions were very common in all other vaccines and could be reduced using a 25-mm needle instead of a 16-mm needle.[29] Systemic adverse effects such as fever, headache, chills, vomiting, and diarrhea were also reported, but severe cases were only reported in few participants in Zhu et al's[10] study, none in Logunov et al's[12] study, and also few in Folegatti et al's[11] study, with none requiring hospitalization. As the proportion of fever increased with dose in Zhu et al's[10] study, the low- and medium-dose vaccines might be better tolerated, although they had a lower efficacy. The remaining studies reported similar adverse events although Sadoff et al[14] found severe complications such as venous thromboembolic events and seizures in some participants. Since the population size was very large (n = 19,630) and these severe adverse events also occurred in the placebo cohort, more investigations are needed to establish whether the complications were caused by the vaccine.[14] Overall, these studies showed that Ad-vaccine had excellent efficacies, good practicality, and tolerable safety, suggesting Ad-vaccine for broad clinical use if more substantial evidence could be completed.

The strength of this review lies in the novelty and urgency of the included studies, especially in the current pandemic situation, and a large number of samples. Furthermore, the immune response mechanism against COVID-19 can be further understood because the efficacy of Ad-vaccines included in this systematic review is built on immune system induction. Ad-vaccines can be a good prevention method in the current conditions as it induces a strong immune response and confers protection against infection. The study limitations include the language restrictions as only studies written in English were reviewed, result reporting, and full-text availability.

Further and more rigorous clinical trials in evaluating the efficacy of Ad-vaccines in numerous subjects are suggested. When the evidence and safety are further established, a review by the authorities might be suitable to confirm whether the use and distribution of Ad-vaccine for COVID-19 could be integrated within the current policies, considering that this vaccine is still relatively effective for the new 20H/501Y.V2 variant.

In conclusion, this systematic review and meta-analysis have proven that Ad-vaccine is effective in inducing the immune system, particularly in the rise of IgG and T-cell responses against SARS-CoV-2 with a pooled OR of 0.26 ($p<0.00001$) and a pooled efficacy of 84.68 ($p<0.00001$). It is also safe with no significant safety issues in preventing COVID-19. However, further studies which will substantiate the evidence for potential clinical implementation are needed to alleviate the burden of COVID-19 globally.

Conflict of Interest
The authors affirm no conflict of interest in this study.

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