MAJOR ARTICLE

Measuring Vaccine Efficacy Against Infection and Disease in Clinical Trials: Sources and Magnitude of Bias in Coronavirus Disease 2019 (COVID-19) Vaccine Efficacy Estimates

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Background. Phase III trials have estimated coronavirus disease 2019 (COVID-19) vaccine efficacy (VE) against symptomatic and asymptomatic infection. We explore the direction and magnitude of potential biases in these estimates and their implications for vaccine protection against infection and against disease in breakthrough infections.

Methods. We developed a mathematical model that accounts for natural and vaccine-induced immunity, changes in serostatus, and imperfect sensitivity and specificity of tests for infection and antibodies. We estimated expected biases in VE against symptomatic, asymptomatic, and any severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections and against disease following infection for a range of vaccine characteristics and measurement approaches, and the likely overall biases for published trial results that included asymptomatic infections.

Results. VE against asymptomatic infection measured by polymerase chain reaction (PCR) or serology is expected to be low or negative for vaccines that prevent disease but not infection. VE against any infection is overestimated when asymptomatic infections are less likely to be detected than symptomatic infections and the vaccine protects against symptom development. A competing bias toward underestimation arises for estimates based on tests with imperfect specificity, especially when testing is performed frequently. Our model indicates considerable uncertainty in Oxford-AstraZeneca ChAdOx1 and Janssen Ad26.COV2.S VE against any infection, with slightly higher than published, bias-adjusted values of 59.0% (95% uncertainty interval [UI] 38.4–77.1) and 70.9% (95% UI 49.8–80.7), respectively.

Conclusions. Multiple biases are likely to influence COVID-19 VE estimates, potentially explaining the observed difference between ChAdOx1 and Ad26.COV2.S vaccines. These biases should be considered when interpreting both efficacy and effectiveness study results.

Keywords. COVID-19; SARS-CoV-2; vaccine efficacy; bias; Clinical Trial.

The coronavirus disease (COVID-19) phase III vaccine trials have demonstrated efficacy against symptomatic infection for multiple vaccines, with estimates ranging from 50% to 95% [1]. Yet a vaccine that protects against symptomatic disease may work by preventing infection (infection-blocking vaccine), by preventing progression to symptoms upon infection (disease-blocking vaccine), or by a combination of these 2 mechanisms (Supplementary Figure 1) [2]. Understanding the extent to which the COVID-19 vaccines protect against infection is important because the success of their vaccination programs

is highly contingent not only on symptomatic cases but also asymptomatic infection and community transmission [3].

The predominant primary outcome of the COVID-19 vaccine trials is vaccine efficacy (VE) against the first case of polymerase chain reaction (PCR)-confirmed symptomatic disease, VE_{sym}. This is measured by PCR-testing trial participants with COVID-19 symptoms and is sensitive to the clinical case definition [4]. As secondary outcomes, most trials also measure the incidence of asymptomatic infections, using either (i) regular swabbing and PCR testing, or (ii) serological testing for anti-nucleocapsid antibodies at prespecified time intervals, which allows seroconversion after infection to be identified for vaccines based on the spike protein (Table 1). Both strategies allow for estimation of VE against asymptomatic infection (VE_{asym}) and VE against any infection (VE_{in}).

VE_{asym} is a complex outcome due to its relationship with the 2 mechanisms of VE. A vaccine that protects only against infection will reduce the number of symptomatic and asymptomatic infections in equal proportions, leading to a positive VE_{asym}. Yet a vaccine

Table 1. Methods for Measuring Vaccine Efficacy Against Infection, Symptomatic Infection and Asymptomatic Infection in the COVID-19 Phase III Trials

Vaccine Efficacy Outcome	Measurement Method	Vaccine[b]	
		Spike Protein	Whole Virus[c]
Symptomatic infection	PCR swabbing upon self-reporting of symptoms	Oxford-AstraZeneca ChAdOx1 Janssen Ad26.COV2.S GRI Sputnik V	Bharat Biotech Covaxin Sinopharm BBIBP-CorV Sinovac CoronaVac
		Pfizer-BioNTechBNT162b2 Moderna mRNA-1273 Novavax NVX-CoV2373	
Asymptomatic infection	Regular PCR swabbing regardless of symptoms	Oxford-AstraZeneca ChAdOx1[a]	Bharat Biotech Covaxin
	Serology testing at prespecified time intervals	Oxford-AstraZeneca ChAdOx1 Janssen Ad26.COV2.S	
		Pfizer-BioNTechBNT162b2 Moderna mRNA-1273 Novavax NVX-CoV2373	
Overall infection	Serologically confirmed asymptomatic infections + PCR-confirmed symptomatic infections	Janssen Ad26.COV2.S Moderna mRNA-1273 Novavax NVX-CoV2373	Sinovac CoronaVac
	Serologically confirmed asymptomatic infections + serologically confirmed symptomatic infections	Oxford-AstraZeneca ChAdOx1 Janssen Ad26.COV2.S Moderna mRNA-1273 GRI Sputnik V Novavax NVX-CoV2373	Sinovac CoronaVac
	PCR-confirmed asymptomatic infections + PCR-confirmed symptomatic infections	Oxford-AstraZenecaChAdOx1	Bharat Biotech Covaxin

Abbreviations: COVID-19, coronavirus disease 2019; GRI, Gamaleya Research Institute; mRNA, messenger RNA; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

[a]COV002 (UK) trial only.

[b]Vaccines only listed where measurement approach has been reported. Trial numbers provided in Supplementary Table 1.

[c]For vaccines based on the whole virus, it may be possible to infer infection via a rise in SARS-CoV-2 antibodies following a period after vaccination.

that protects against symptom development will convert symptomatic cases to asymptomatic, potentially giving a negative VE_{asym}. The counterintuitive interpretation of this outcome has been noted [2, 5], but the relationship between VE_{asym}, VE_{in}, and VE against progression to symptoms (VE_{pr}) has not been quantified.

Estimates of VE are known to be biased by factors such as imperfect test sensitivity and specificity and the accumulation of immunity over time [6–8]. However, there has been little discussion on the potential biases of the COVID-19 VE estimates [9, 10]. We developed a mathematical model of a vaccine trial to investigate the factors affecting observed values of VE. We first illustrate parameters affecting measured VE_{asym}, then quantify the influence of different biases on VE estimates, notably the impact of (i) the build-up of immunity from undetected asymptomatic infections, (ii) imperfect test sensitivity and specificity for alternative testing strategies, (iii) differential detection of asymptomatic and symptomatic infections, and (iv) confounding of VE and probability of symptoms by age. We finish by estimating bias-adjusted VEs for 2 COVID-19 vaccines.

METHODS

Analytical Derivations

VE is defined as 1-RR, where RR is some measure of the relative risk in the vaccine compared with the control arm [11]. For most primary and secondary outcomes of the COVID-19 vaccine trials, the relative risk is based on an incidence rate ratio (IRR) such that

$$VE = 1 - IRR = 1 - \frac{\frac{number\ outcomes\ (vaccine)}{person\text{-}years\ observation\ (vaccine)}}{\frac{number\ outcomes\ (control)}{person\text{-}years\ observation\ (control)}} = 1 - \frac{IR_v}{IR_c}$$

where IR_v and IR_c are the incidence rate in the vaccine and control groups respectively.

For outcomes measured at fixed time points (eg, seroconversions), the relative risk can be calculated using the cumulative incidence ratio (CIR) such that

$$VE = 1 - CIR = 1 - \frac{\frac{cumulative\ number\ seroconverted\ (vaccine)}{number\ tested\ (vaccine)}}{\frac{cumulative\ number\ seroconverted\ (control)}{number\ tested\ (control)}} = 1 - \frac{CI_v}{CI_c}$$

where CI_v and CI_c are the cumulative incidence in the vaccine and control groups respectively. For a "leaky" vaccine, VE based on cumulative incidence approximates that based on the incidence rate for low incidence or short follow-up

periods but biases toward zero as follow-up time and incidence increase [8].

For vaccines that protect against infection and/or symptoms, VE against symptomatic infection is given by

$$VE_{sym} = 1 - (1 - VE_{in})(1 - VE_{pr}) \quad (1)$$

[12]. VE against asymptomatic infection depends on the incidence of asymptomatic infections that are not prevented by the vaccine and on symptomatic infections that the vaccine prevents from progressing, such that

$$VE_{asym} = 1 - \frac{(1 - p_s)(1 - VE_{in}) + p_s(1 - VE_{in})VE_{pr}}{(1 - p_s)} \quad (2)$$

where p_s is the probability of symptoms in the absence of vaccination. Substituting equation 1 into equation 2 allows VE_{in} to be derived as a simple function of VE_{sym} and VE_{asym}

$$VE_{in} = (1 - p_s)VE_{asym} + p_s VE_{sym} \quad (3)$$

Rearranging equation 1 and substituting equation 3 into equation 1 then provides a solution for VE_{pr}

$$VE_{pr} = 1 - \frac{1 - VE_{sym}}{1 - ((1 - p_s)VE_{asym} + p_s VE_{sym})} \quad (4)$$

If asymptomatic infections are less likely to be detected than symptomatic infections, and a vaccine is protective against symptom development ($VE_{pr} > 0$), then observed $VE_{in} \neq$ true VE_{in}. The observed VE_{in} depends on the relative incidence of detected infections, and can be related to the true efficacy by

$$Observed\ VE_{in} = VE_{in} + \frac{p_s(1 - \sigma)(1 - VE_{in})VE_{pr}}{1 - (1 - p_s)(1 - \sigma)} \quad (5)$$

where σ represents the relative probability of asymptomatic to symptomatic infection detection. For intermediate steps for equations 3–5 and estimation of confidence intervals for VE_{in} and VE_{pr}; see Supplementary Methods.

Analytical solutions become more complex when incorporating additional biases, so we developed a stochastic mathematical (cohort) model of a phase III vaccine trial.

Mathematical Model

The model follows a susceptible, infected, recovered (SIR) structure, implemented as a Markov model, and allows for asymptomatic and symptomatic infections, natural immunity, changes in serostatus and imperfect test sensitivity and specificity. We assume a constant infection rate over time and a "leaky vaccine" model, so each vaccinated individual's probability of infection is reduced by VE_{in} and their risk of then developing symptoms is reduced by VE_{pr}. We assume no heterogeneity in population characteristics but perform a sensitivity analysis to assess the effect of variation in p_s and VE by age.

We model 2 testing approaches for asymptomatic infections: (i) weekly PCR testing and (ii) serological testing at 1, 2, 6, 12, and 24 months after baseline. We assume that responsive PCR

testing detects all symptomatic infections. Observed VE is calculated from the simulated incidence of detected infections in each trial arm. Efficacy is estimated as 1-IRR for all outcomes except those estimated using serology, for which efficacy is estimated as 1-CIR, using the cumulative number of seroconversions detected in each serology assessment up to the present time interval. Point estimates and confidence intervals are given by the mean and 2.5 and 97.5 percentiles of 1000 simulated estimates.

Application to COVID-19

Applying the model to COVID-19, we assumed a natural probability of developing symptoms upon infection of 0.67 [13], a serology test specificity of 99.84% [14], and sensitivity of 95% and 80% to symptomatic and asymptomatic infections, respectively [15]. We used data on the probability of PCR detection over time since infection for individuals without symptoms [16] to estimate the probability of detecting an asymptomatic infection with weekly PCR swabbing (Supplementary Table 2) and assumed a PCR test specificity of 99.945% [17]. We used the model to estimate bias-adjusted VE estimates for 2 adenovirus vector vaccines with published trial data on asymptomatic infection, ChAdOx1 (Oxford-AstraZeneca) and Ad26.COV2.S (Janssen). We used our best parameter estimates to estimate the infection rate from the number of reported infections in the placebo arm, accounting for imperfect test characteristics. We ran the model under a range of true VE_{in} and VE_{pr} values to find which combination gave the trial-reported estimates, then generated 95% uncertainty intervals (UI) using Latin hypercube sampling to give the range within which the VE is expected to lie, considering both statistical variation and parameter uncertainty. We then used rank regression to evaluate the contribution of individual parameters to the biases (Supplementary Methods).

The model is described further in Supplementary Methods and Supplementary Figures 2 and 3. Model parameters are provided in Supplementary Table 3. Code is available at: https://github.com/lucyrose96/COVID-19-Trial-Model.

RESULTS

Interpretation of Vaccine Efficacy Against Asymptomatic Infection

Observed VE_{asym} was positively associated with VE_{in} but negatively associated with VE_{pr} and the proportion of infections that were symptomatic (Figure 1). For vaccines that only prevented infection, VE_{asym} was equal to VE_{sym}. For vaccines with efficacy predominantly mediated by prevention of symptoms, VE_{asym} was low or negative, particularly when a large proportion of infections were naturally symptomatic. For vaccines with high VE_{sym} (Figure 1A), protection against infection can be expected with lower values of VE_{asym} than vaccines with moderate VE_{sym} (Figure 1B). VE_{in} and VE_{pr} could be estimated
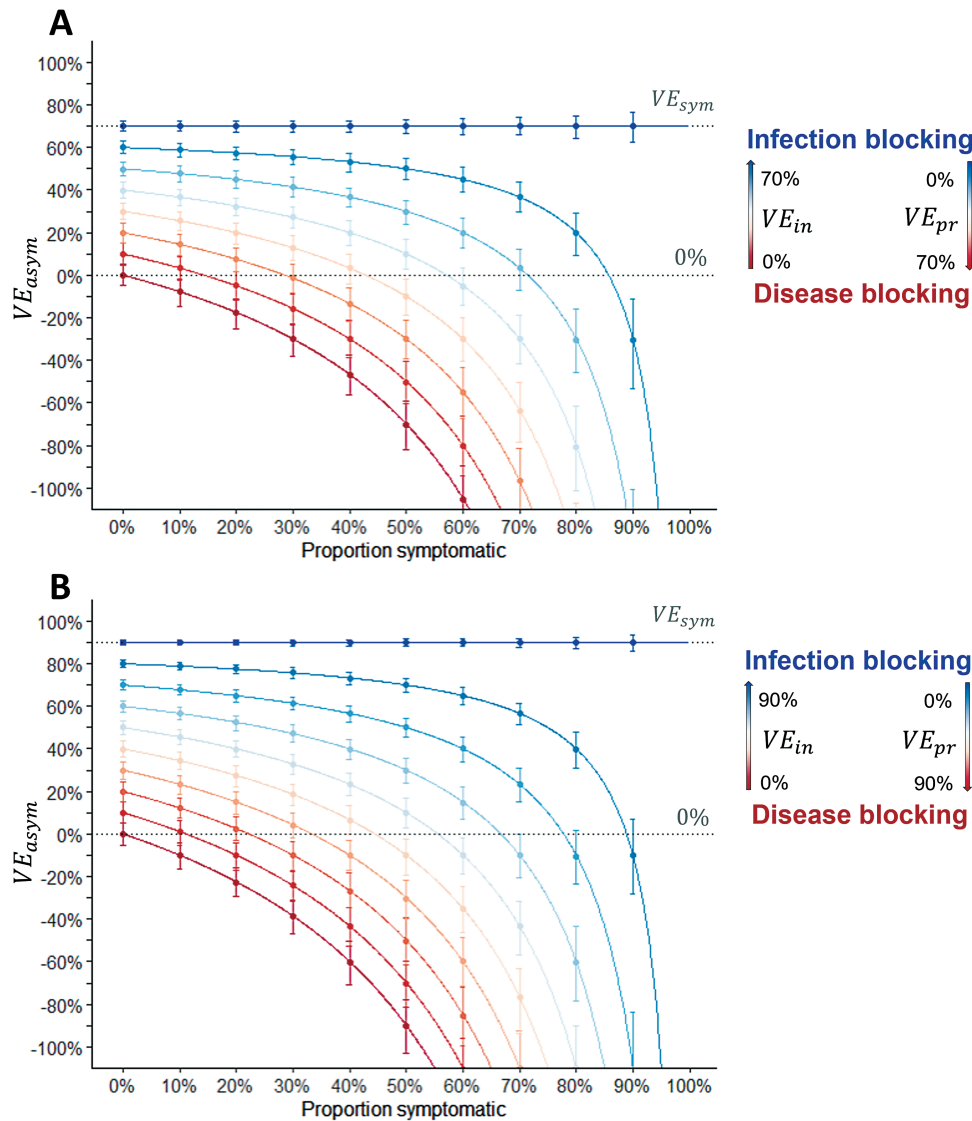
Figure 1. Estimated vaccine efficacy against asymptomatic infection. Red to blue gradient represents the transition from a disease-blocking vaccine (VE_{in} = 0% and VE_{pr} = VE_{sym}) to an infection-blocking vaccine (VE_{in} = VE_{sym} and VE_{pr} = 0%). Each successive line represents a 10% increase in VE_{in} and a corresponding decrease in VE_{pr} (such that VE_{sym} remains the same). Lines show predicted values from equation 2; points and error bars show the observed mean and 2.5 and 97.5 percentiles from 1000 simulations, with efficacy calculated as 1-IRR, censoring after the first infection. *A*, Vaccine with 70% efficacy against symptomatic infection (eg, Oxford-AstraZeneca/Janssen vaccines). *B*, Vaccine with 90% efficacy against symptomatic infection (eg, Pfizer/Moderna vaccines). Abbreviations: IRR, incidence rate ratio; VE_{asym}, vaccine efficacy against asymptomatic infection; VE_{in}, vaccine efficacy against infection; VE_{pr}, vaccine efficacy against progression to symptoms; VE_{sym}, vaccine efficacy against symptomatic infection.

from VE_{sym} and VE_{asym} using equations 3 and 4 (Supplementary Figure 4).

Possible Biases in COVID-19 Vaccine Trials

The build-up of immunity from undetected asymptomatic infections caused VE_{sym} to bias in opposite directions for infection-blocking and disease-blocking vaccines. For infection-blocking vaccines, estimated VE_{sym} decreased over time, with greater decreases observed for higher infection rates and lower probabilities of symptoms (Figure 2A). For disease-blocking vaccines, a downward bias was only observed when the probability of symptoms was low (Figure 2C). Instead, for most combinations

of parameters, estimated VE_{sym} increased slightly over time. For an infection and disease-blocking vaccine (50% VE_{in}, 40% VE_{pr}), a small downward bias was observed (Figure 2B). The biases were sensitive to the VE calculation, as VE_{sym} estimated with cumulative incidence decreased over time for all vaccine profiles (Supplementary Figure 5).

Imperfect test characteristics biased efficacy estimates toward zero. Factors increasing the magnitude of the bias were: reduced specificity, reduced sensitivity, increased testing frequency, and calculation with the CIR instead of the IRR. Although the serology estimated VE_{in} was based on the CIR (as person-time at risk is unknown), the bias was usually lower than the
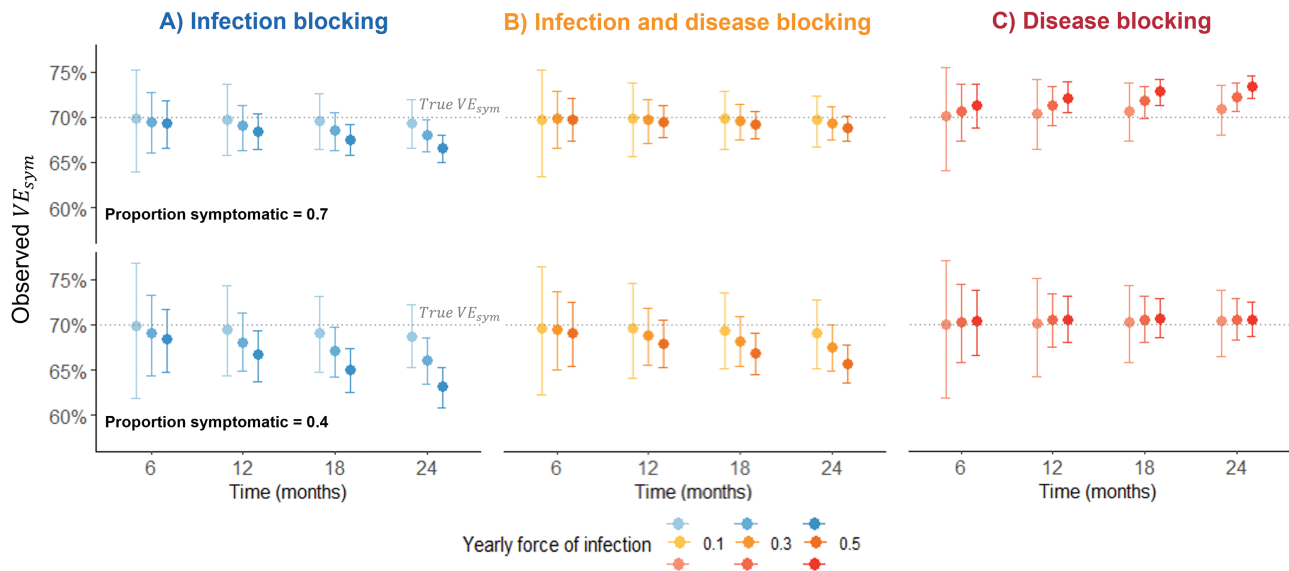
Figure 2. Change in estimated vaccine efficacy against symptomatic infection over a 2-year follow-up. Points and error bars show the observed mean and 2.5 and 97.5 percentiles from 1000 simulations, with efficacy calculated as 1-IRR, censoring after the first symptomatic infection. Time = 0 months represents 2 weeks post second dose. Sensitivity and specificity = 100%. True vaccine efficacies: VE_{sym} = 70%. *A*, VE_{in} = 70%; VE_{pr} = 0%. *B*, VE_{in} = 50%, VE_{pr} = 40%. *C*, VE_{in} = 0%, VE_{pr} = 70%. Abbreviations: IRR, incidence rate ratio; ; VE_{asym}, vaccine efficacy against asymptomatic infection; VE_{in}, vaccine efficacy against infection; VE_{pr}, vaccine efficacy against progression to symptoms; VE_{sym}, vaccine efficacy against symptomatic infection.

weekly-PCR estimate, for a given sensitivity and specificity, due to the lower frequency of testing (Figure 3). This led to substantial bias particularly in low incidence settings. For example, with a high specificity (99.8%) and sensitivity (100%), a true VE_{in} of 70% in a low incidence setting (5% per year) was underestimated at 23%.

For a vaccine that was protective against symptom development ($VE_{pr} > 0$), VE_{in} was overestimated when asymptomatic infections were less likely to be detected than symptomatic infections (Figure 4). The greater the difference in the detection probabilities and the greater the vaccine's protection against symptoms, the greater the overestimation.

These results were insensitive to adding age stratification to the probability of symptoms. However, also adding age-stratification to VE led to biased VE_{sym} and VE_{asym} estimates, when not adjusted for age (Figure 5). When VE decreased with age and the probability of symptoms increased, VE_{asym} was overestimated and VE_{sym} underestimated, while the opposite was observed when efficacy increased with age. The magnitude of the difference was greater with an increased association between age and the probability of symptoms, and between age and efficacy.

Estimating VE_in, VE_pr, and the Likely Bias From the Published Trial Results
Applying equations 3 and 4 to the reported trial results gave an estimated VE_{pr} for ChAdOx1 of 43.6% (95% confidence interval [CI] 20.6–59.9) (Table 2). For Ad26.COV2.S, VE_{in} was estimated at 66.2% (95% CI 55.9–74.1), and VE_{pr} just 0.9% (95% CI −46.8 to 33.2).

Incorporating the aforementioned biases, the model gave best estimates for ChAdOx1 VE_{in}, VE_{asym} and VE_{pr} of 59.0% (95% UI 38.4–77.1), 27.4% (95% UI −32.1 to 87.4) and 31.5% (95% UI −20.7 to 55.0) respectively. While, for Ad26.COV2.S, the corresponding bias-adjusted estimates were 70.9% (95% UI 49.8–80.7), 79.2% (95% UI 14.6–99.0). and −15.2% (95% UI −73.3 to 33.2). The biases were most sensitive to the test specificity, infection rate, and testing adherence (Supplementary Tables 4–6).

DISCUSSION

Accurately estimating COVID-19 VE outcomes is important to understand vaccine benefits, their likely impact on transmission and the long-term prospects for disease control. Simulating a COVID-19 vaccine trial helps to characterize the likely influence of biases and may help to explain differences seen between vaccines, trials, and populations.

We first derived the relationship between VE_{asym} with efficacy against infection and against disease in breakthrough infections. While increasing VE_{in} increased VE_{asym}, increasing VE_{pr} decreased VE_{asym} because more infections were prevented from becoming symptomatic. This influence of VE_{pr} was stronger when the probability of symptoms was higher. Therefore, although counterintuitive, for COVID-19 where a minority of infections present asymptomatically and the vaccines have high efficacies against symptomatic infection, protection against infection can be expected even when VE_{asym} is low or negative. A vaccine with a high VE_{asym} would work predominantly by
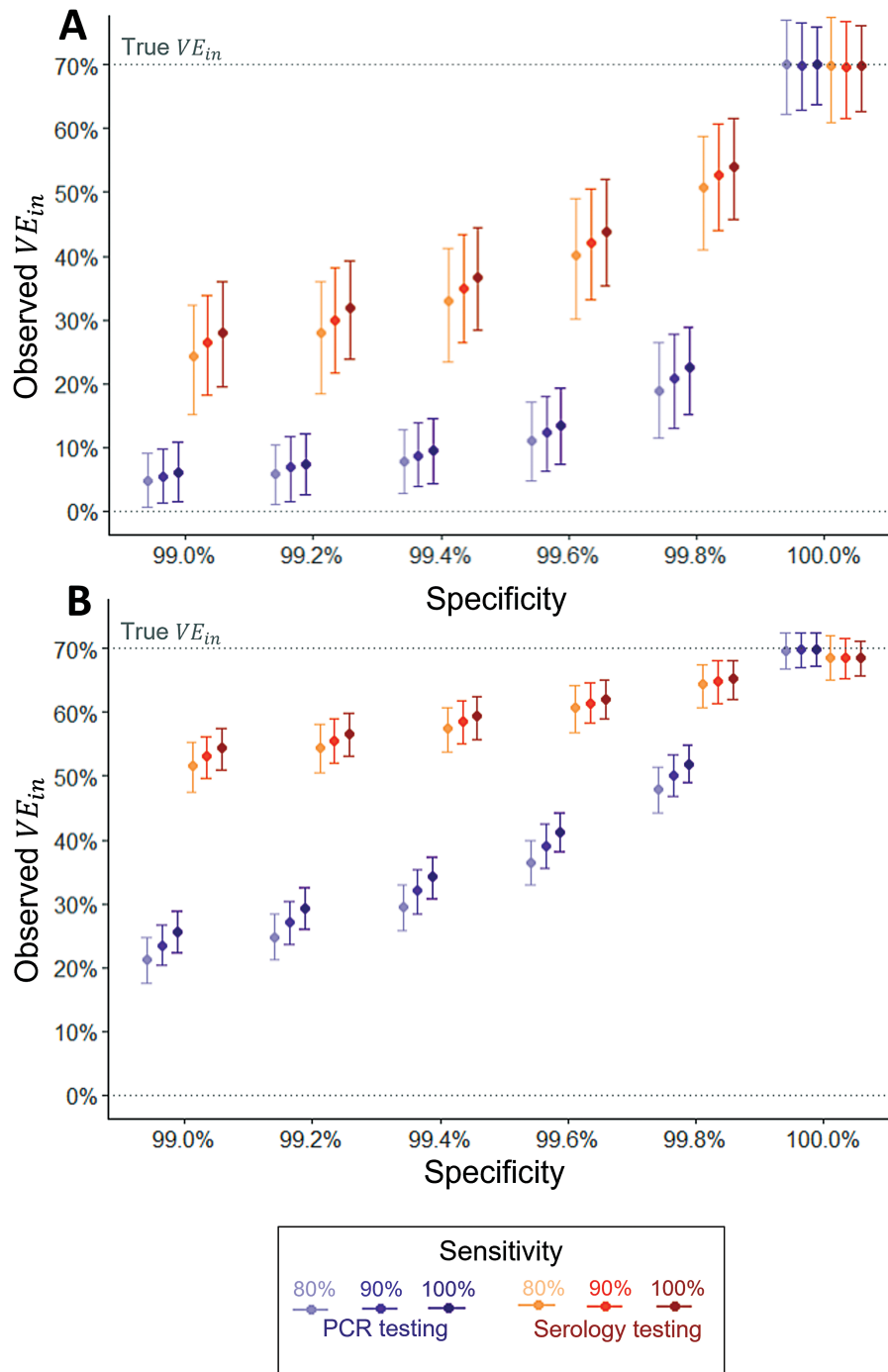
Figure 3. Impact of imperfect test sensitivity and specificity on serology-estimated and PCR-estimated vaccine efficacy against infection. *A*, Low force of infection (5% per year). *B*, High force of infection (30% per year). At 6-month follow-up visit: serology tests taken at month 1, 2, and 6 (cumulative seroconversions up to 6-month visit); PCR tests taken weekly. Serology efficacy calculated using 1-CIR; PCR efficacy calculated using 1-IRR. Sensitivity assumed to be equal for symptomatic and asymptomatic infections. Points and error bars represent the mean and 2.5 and 97.5 percentiles from 1000 simulations. At 100% specificity, a slight bias is observed for the serology-estimated VE_{in} because estimates based on the CIR bias toward zero over time, particularly in high incidence settings [8]. Abbreviations: CIR, cumulative incidence ratio; IRR, incidence rate ratio; PCR, polymerase chain reaction; VE_{in}, vaccine efficacy against infection.

preventing infection (high VE_{in}, low VE_{pr}), whereas a vaccine with a low VE_{asym} would work predominantly by preventing symptoms (low VE_{in}, high VE_{pr}).

Second, we estimated that the ChAdOx1 weekly PCR-measured VE_{in} was underestimated by 8.1% (Trial 50.9%,

Model 59.0%) and VE_{asym} by 12.8% (Trial 14.6%, Model 27.4%). The VE_{pr} calculated from the trial reported VE_{in} and VE_{sym} would therefore be an overestimation (Calculation 43.6%, Model 31.5%). However, a wide range of values are compatible with the reported trial results when considering stochastic
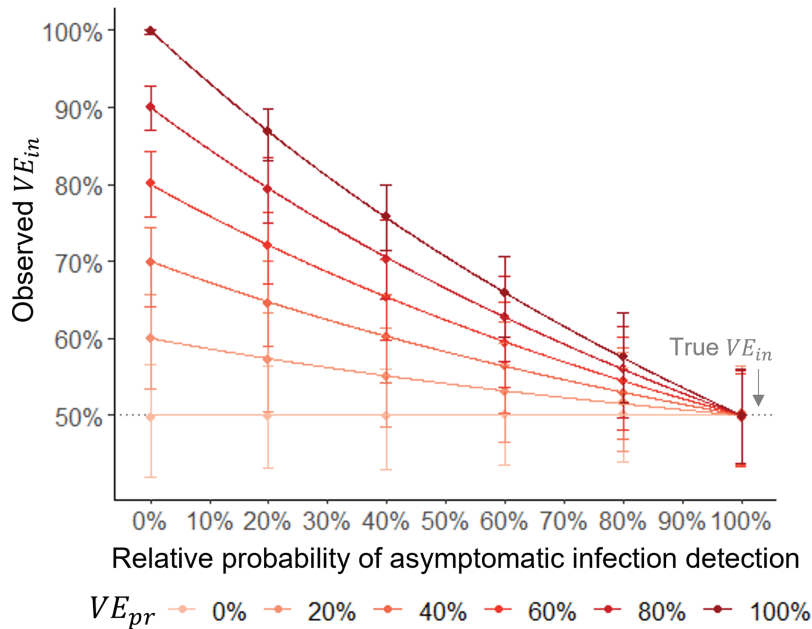
Figure 4. Impact of differential detection of asymptomatic and symptomatic infections on observed vaccine efficacy against infection. Lines show values estimated with equation 4, points and error bars show the observed mean and 2.5 and 97.5 percentiles from 1000 simulations, with efficacy calculated as 1-IRR, censoring after the first infection. Specificity = 100%, yearly force of infection = 5%, follow-up = 12 months from 2 weeks post 2nd dose. Abbreviations: IRR, incidence rate ratio; VE_{in}, vaccine efficacy against infection; VE_{pr}, vaccine efficacy against progression to symptoms.

variation and parameter uncertainty. The small sample size informing the Ad26.COV2.S VE_{asym} estimate and parameter uncertainty for the test specificity, infection rate and adherence to PCR testing, in particular, reduced the precision of
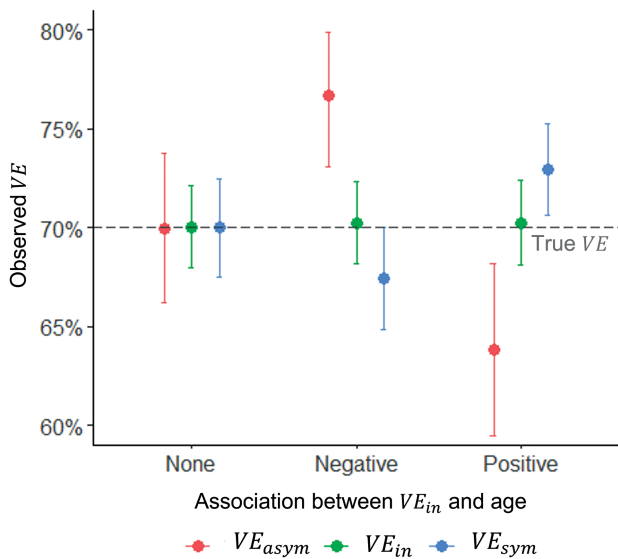
Figure 5. Estimated vaccine efficacy in a population with a higher probability of symptoms with age. Points and error bars represent the mean and 2.5 and 97.5 percentiles of 1000 simulations. True VE_{asym} = true VE_{in} = true VE_{sym} = 70%. Specificity = 100%, yearly force of infection = 20%, follow-up = 12 months from 2 weeks post 2nd dose. Abbreviations: VE_{asym}, vaccine efficacy against asymptomatic infection; VE_{in}, vaccine efficacy against infection; VE_{sym}, vaccine efficacy against symptomatic infection

our uncertainty intervals. The true VE_{in} may range between 38.4% and 77.1%, and VE_{pr} between −20.7% and 55.0%. Given the strong bias that can be caused by reduced test specificity and a high frequency of testing, it would not be unreasonable for the true VE_{in} to be closer to our upper uncertainty interval, especially considering that effectiveness studies have estimated greater protection against infection than the trial [27, 28]. For Ad26.COV2.S, our model suggests that the true VE_{in} lies between 49.8% and 80.7%, with a best estimate of 70.9%. Although this indicates a negative VE_{pr}, we believe this is unlikely and rather explained by the small sample size informing the trial VE_{asym} estimate.

We explain these overall expected differences by 4 likely biases acting in the COVID-19 trials.

1. A lower probability of detecting asymptomatic infections relative to symptomatic infections leads to overestimation of VE_{in} if the vaccine protects against symptom development. For these vaccines, some infections will be prevented from causing symptoms, so will be less likely to be detected. VE_{pr} would be mistaken for VE_{in}, so VE_{in} would be overestimated. Both conditions for this bias are likely to be satisfied in the COVID-19 trials, as virological and serological testing approaches are less sensitive to asymptomatic infections [29, 30]. This bias is likely to have influenced the ChAdOx1 VE_{in} estimate, however we expect it was overridden by a competing downward bias. It is important to note that this bias does not affect VE_{asym} or VE_{sym}.

Table 2. Estimated Vaccine Efficacy Against Infection (VE_{in}) and Vaccine Efficacy Against Progression to Symptoms (VE_{pr}) Using Trial-reported Vaccine Efficacy Against Symptomatic Infection (VE_{sym}) and Vaccine Efficacy Against Asymptomatic Infection (VE_{asym})

Vaccine	Reported Vaccine Efficacy (%)			Calculated Vaccine Efficacy (%)		Bias Adjusted Vaccine Efficacy (%)		
	VE_{sym}	VE_{asym}	VE_{in}	VE_{in}	VE_{pr}	VE_{asym}	VE_{in}	VE_{pr}
Oxford-AstraZeneca ChAdOx1[a] [18]	72.3	14.6	50.9	…	43.6	27.4	59.0	31.5
	63.1 to 79.3	−12.1 to 34.9	41.0 to 59.0		20.6 to 59.9	−32.1 to 87.4	38.4 to 77.1	−20.7 to 55.0
Janssen Ad26.COV2.S[b,c] [19]	66.5	65.5	…	66.2	0.9	79.2	70.9	−15.2
	55.5 to 75.1	39.9 to 81.1		55.9 to 74.1	−46.8 to 33.2	14.6 to 99.0	49.8 to 80.7	−73.3 to 33.2
Pfizer-BioNTech BNT162b2[d] [20]	95.0	…	…	…	…	…	…	…
	90.3 to 97.6							
Moderna mRNA-1273[d] [21]	94.1	…	…	…	…	…	…	…
	89.3 to 96.8							
Novavax NVX-CoV2373[d] [22]	96.4	…	…	…	…	…	…	…
	73.8 to 99.5							
Sinovac CoronaVac[d] [23]	83.5	…	…	…	…	…	…	…
	65.4 to 92.1							
Sinopharm BBIBP-CorV[d] [24]	78.1	…	…	…	…	…	…	…
	64.8 to 86.3							
Gamaleya Research Institute Sputnik V[d] [25]	91.6	…	…	…	…	…	…	…
	85.6 to 95.2							
Bharat Biotech Covaxin[d] [26]	80.6	…	…	…	…	…	…	…
	…							

Trials that have not yet reported an estimate are left blank. VE_{in} and VE_{pr} calculated using equations 3 and 4, respectively.

Abbreviations: mRNA, messenger RNA; PCR, polymerase chain reaction; VE_{asym}, vaccine efficacy against asymptomatic infection; VE_{in}, vaccine efficacy against infection; VE_{pr}, vaccine efficacy against progression to symptoms; VE_{sym}, vaccine efficacy against symptomatic infection..

[a] VE_{sym} measured with responsive PCR testing of symptomatic participants, VE_{asym} measured using weekly PCR testing of asymptomatic participants, VE_{in} estimated from all positive PCR tests collected via the alternative testing strategies for symptomatic and asymptomatic infections.

[b] VE_{sym} measured with responsive PCR testing of symptomatic participants, VE_{asym} measured using serology testing of asymptomatic participants.

[c] VE_{in} and VE_{pr} based on odds ratio, assuming confidence intervals are normal on the log scale.

[d] VE_{sym} measured with responsive PCR testing of symptomatic participants.

2. Imperfect test sensitivity and specificity bias estimates toward zero, with greater bias with higher frequency of testing, lower infection rate and for VE based on cumulative incidence rather than incidence rates. This bias is caused by false positives in both trial arms and is greater with higher ratios of false positives to true positives [6]. It has potential to affect all VE outcomes but is likely to affect estimates of VE_{asym} and VE_{in} more than VE_{sym}, because the combined probability of experiencing symptoms consistent with COVID-19 when not infected with SARS-CoV-2 and receiving a false positive test is low. Regression analysis showed that this was the predominant factor leading to underestimation of VE_{asym} and VE_{in} for both ChAdOx1 and Ad26.COV2.S in our model. As the bias is greater when testing is frequent, even a test with high specificity could bias the estimated ChAdOx1 VE_{in} and VE_{asym} noticeably toward zero. This could explain such contrasting trial reported VE_{asym} estimates between ChAdOx1 and Ad26.COV2.S, despite their similar platforms and neutralizing antibody responses [31, 32].

3. A build-up of natural immunity from undetected asymptomatic infections contributes a small downward bias in VE_{sym} for infection-blocking vaccines and a small upward bias for disease-blocking vaccines. For an infection-blocking vaccine, the proportion of infections that are asymptomatic is unaltered by the vaccine. Therefore, the rate at which immunity from asymptomatic infections accumulates, relative to the detection of symptomatic infections, is equivalent across trial arms, leading to an underestimation of VE_{sym} [8, 33]. Yet for disease-blocking vaccines, a greater proportion of infections in the vaccine arm will be asymptomatic, accelerating the acquisition of immunity from undetected infections and introducing a conflicting upward bias. Our model and real-world effectiveness studies suggest the COVID-19 vaccines protect against both infection and symptoms [27, 34]. Therefore we expect the overall direction of this bias to be toward zero, and for its magnitude to be greater for vaccines with higher VE_{in}.

4. Decreasing VE by age will bias estimated VE_{sym} downward and VE_{asym} upward, unless adjusted for age. This is due to older participants contributing more to VE_{sym} estimates than younger participants, who contribute more to VE_{asym} estimates. This bias is dependent on the probability of symptoms increasing with age, for which there is mixed evidence [35–37]. However, it should be considered when interpreting estimates based on different subgroups, such as VE_{asym} estimates based on a subgroup with serological data when VE_{sym} is based on the total population.

These biases also apply to effectiveness studies, based on cohort or case-control designs. Notably, the bias arising from differential

detection of asymptomatic and symptomatic infections will likely be greater in real-world studies, where asymptomatic testing is less rigorous. This should be considered when comparing real-world and trial reported estimates, as it could lead to greater bias toward overestimation of VE_{in} in effectiveness studies.

Limitations to our analysis include uncertainties over parameter estimates. There is limited evidence on serology and PCR test sensitivities for asymptomatic infections, and how these change over time. As we show, differences in test sensitivity by symptom status can lead to overestimation of VE_{in}, so further studies are needed to clarify the potential role of this bias. We also did not consider the vaccines' effects on viral load and how this alters virological and serological test sensitivity. Multiple COVID-19 vaccines reduce SARS-CoV-2 viral load [18, 38], and lower load infections are less likely to lead to seroconversion [39]. Therefore serology-based efficacy estimates may be more representative of high viral load infections than all infections, and may be comparable to estimates based on DNA sequenced swabs, which must exceed a threshold viral load to be sequenced. Finally, we do not consider point prevalence estimates from single time point PCR swabs, however this has been explored elsewhere [9, 10].

In conclusion, multiple biases have the potential to influence COVID-19 VE estimates, with their direction and magnitude dependent on the vaccine properties and testing strategies. These biases may explain differences between the ChAdOx1 and Ad26.COV2.S trial estimates despite similar vaccine platform technologies, and should be considered when interpreting efficacy and effectiveness study results as they are reported for these and other vaccines.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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