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Comparison of methodological approaches in COVID-19 vaccine effectiveness estimation using observational data

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Comparison of methodological approaches in COVID-19 vaccine effectiveness estimation using observational data

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ABSTRACT

Background: Target trial emulation (TTE) is a framework to systematically address potential design-related biases in causal inference when using observational data. We estimated vaccine effectiveness (VE) of the Omicron XBB.1.5 booster vaccination against SARS-CoV-2 infection between 2 October 2023 and 2 April 2024 using four TTE approaches.

Methods: A hypothetical target trial was designed where eligible participants would be randomly assigned to receive booster vaccination or not. Four approaches were used to emulate this hypothetical trial using data of an ongoing prospective cohort study in the Netherlands. The first and second approach defined time zero as the start of the booster vaccination rollout and considered vaccination as a time-varying variable. The first approach adjusted for confounders by regression adjustment, while the second used inverse probability weighting. The third and fourth approach used multiple time zeros. In the third approach, all persons who received a booster vaccination on a specific day were matched 1:1 to persons who were not (yet) vaccinated on that day. In the fourth approach, confounders were adjusted for using inverse probability weighting.

Results: Overall VE was 30% (95%CI:26-34), 28% (24-32), 25% (18-30) and 27% (24-31) in the first, second, third and fourth approach, respectively. VE decreased as time since vaccination increased.

Conclusions: Estimated VE was similar across the four approaches. The choice of approach should be based on the model assumptions, estimand of interest, and feasibility.

KEYWORDS

Cohort studies, Coronavirus Disease 2019 (COVID-19), Causal Inference, Vaccination, Target Trial Emulation

BACKGROUND

In epidemiology, causal questions, primarily questions about comparing effectiveness or safety of interventions, are ideally answered using an appropriately designed and conducted randomized trial. However, these trials are often not feasible, not ethical, or time-consuming. Furthermore, external validity is often limited. For COVID-19 vaccines, some important questions regarding vaccine effectiveness (VE) after the initial roll-out could not be answered by randomized trials. In particular, VE in specific subpopulations, VE against newly emerging variants, duration of protection, and the benefit of booster vaccinations could not be assessed in trials [1]. For these questions, public health policy relies on observational studies. However, the nature of observational data poses several challenges for causal inference, including confounding bias, selection bias, and immortal time bias [2-4].

To help reduce design-related errors that can result in biased estimates and erroneous causal conclusions, one can use "target trial emulation" (TTE) [5]. TTE is a framework for designing and analyzing observational studies that aim to estimate a causal effect. The systematic approach of TTE consists of two steps: 1) specify a hypothetical randomized trial that would answer the causal question, and 2) specify how each element of this trial was emulated using observational data [6]. The hypothetical randomized target trial should be reasonably possible to emulate with the available observational data, rather than disregard any constraints. The protocol must include certain key elements that define the causal estimand, which is the description of the exact treatment effect under study, and the data analysis plan. The key elements are the eligibility criteria, treatment strategies, treatment assignment, the start and end of follow-up, outcomes, causal contrasts.

While TTE does not eliminate bias from inadequate data, it helps to remove design biases if the observational study protocol properly emulates the hypothetical randomized target trial that would answer the causal question. To ensure this, three components should be aligned at time zero (baseline): eligibility criteria are met, treatment strategies are assigned, and follow-up is started. For

this, a key challenge is non-unique time zero, i.e. a single person may meet the eligibility criteria at multiple times, for instance throughout a vaccination campaign [7]. To ensure alignment at time zero, one could choose a single time zero, for example the first eligible time, or use every eligible time as time zero. The latter requires emulating multiple sequential target 'trials', each of them with a different start of follow-up [7].

TTE has become increasingly popular in post-marketing vaccine evaluation in recent years, particularly following the introduction of COVID-19 vaccines [8, 9]. As TTE does not stipulate which specific analytical and statistical methods should be used, the VE studies that applied the TTE framework featured various analytical approaches and statistical methods [8, 9]. Nonetheless, single and multiple time zero(s) approaches can be distinguished and several commonly used methods can be identified, although variation remains between studies that use each of these. Most common for VE studies is sequential target trial emulation with matching of unvaccinated to vaccinated participants [8]. Inverse probability weighting (IPW) is also frequently applied, both in single and multiple time zero(s) approaches [1, 10, 11].

In the Netherlands, a COVID-19 booster vaccination campaign started on October 2, 2023. In this campaign, persons at increased risk for severe COVID-19 were eligible for vaccination: persons ≥60 years, health care workers, and medical risk groups. Everyone vaccinated during this campaign received a monovalent vaccine targeting the SARS-CoV-2 Omicron XBB.1.5 subvariant, irrespective of their vaccination history, and the Comirnaty mRNA (BioNTech-Pfizer, Germany/United States) was used almost exclusively [12].

The aim of this study was to assess real-world VE of the Omicron XBB.1.5 booster vaccination using the TTE framework and using different design and analysis approaches, explain their differences, and assess variability in the obtained estimates.

METHODS

Study design

The VAccine Study COvid-19 (VASCO) is a population-based prospective cohort study that was initiated when COVID-19 vaccination was introduced in the Netherlands [13]. The primary study objective is to estimate COVID-19 VE against SARS-CoV-2 infection. Study details are described elsewhere [13]. Briefly, over 45,000 community-dwelling adults aged 18-85 years were included in VASCO between May and December 2021. Participants are followed for five years after enrollment and data is collected using online questionnaires and self-collected fingerprick blood samples for SARS-CoV-2 serology, both at scheduled intervals. Questionnaires included, among others, questions on sociodemographic factors, COVID-19 vaccination, and SARS-CoV-2 testing results. Participants could also notify COVID-19 vaccinations and positive SARS-CoV-2 tests at any time during follow-up in the study application. SARS-CoV-2 self-tests are provided to participants to facilitate testing in case of COVID-19-like symptoms.

Written informed consent was obtained from all participants prior to enrolment into the study. The VASCO study is conducted in accordance with the principles of the Declaration of Helsinki and the study protocol was approved by the Medical Ethics Committee of the *Stichting Beoordeling Ethiek Biomedisch Onderzoek*, Assen, the Netherlands.

Study population, exposure, outcome and covariates

The *study population* included VASCO study participants vaccinated with the COVID-19 primary series and at least one booster dose who were eligible for the COVID-19 XBB.1.5 booster vaccination, specifically: persons aged over 60 years, healthcare workers and those belonging to a medical risk group. COVID-19 vaccination was based on vaccinations registered in the Dutch national COVID-19 vaccination Information and Monitoring System, supplemented with self-reported

vaccinations. How these data sources were combined is described elsewhere [13]. Booster vaccination (*the exposure*) was defined as receipt of any SARS-CoV-2 vaccination between 2 October 2023 and 2 April 2024, the study period. SARS-CoV-2 infection (*the outcome*) was defined as a self-reported positive SARS-CoV-2 test, or positive serology (seroconversion or a 4-fold increase in antibodies against the nucleoprotein, N-antibodies, detected in two consecutive serology samples). In case of a serology-based infection (i.e. no corresponding infection reported), the infection date was imputed between the before and after infection detection sampling dates. Dates were imputed accounting for SARS-CoV-2 incidence over time based on reported positive SARS-CoV-2 tests in the VASCO population per age group (18-39, 40-59, 60-69, 70-85 years) per day, such that the probability of selecting a date was proportional to the incidence in these age groups. *Covariates* were age group (18–39, 40–59, 60–69, 70–85), sex (male, female, other), educational level, presence of a medical risk condition and time since prior infection. Educational level was classified as low (no or primary education), intermediate (secondary school or vocational training), high (bachelor's degree or higher) or other, based on baseline data. 'Other' was included as an answer option for sex and education level in the questionnaires to accommodate participants whose situation did not fit any of the provided options. Presence of a medical risk condition was defined as having one or more of following conditions: diabetes mellitus, lung disease or asthma, asplenia, cardiovascular disease, immune deficiency, cancer, liver disease, neurological disease, renal disease, organ or bone marrow transplantation. Time since prior infection was defined as time since most recent prior infection before 2 October 2023 (start study period), was based on self-reported positive SARS-CoV-2 tests and N-antibodies detected in serology samples (as described above), and was classified as no prior infection, a prior infection ≥1 year ago or a prior infection <1 year ago. The covariates age, presence of a medical risk condition and time since prior infection were time-varying. During follow-up, age increased and presence of a medical risk condition could switch from no to yes if a medical condition was reported in a follow-up questionnaire. Time since prior infection could switch from prior infection <1 year ago to prior infection ≥1 year ago. Participants could not switch from no prior

infection to prior infection <1 year ago, as infections during follow-up were considered outcomes and such participants were no longer eligible for vaccination within the vaccination campaign period.

Methodological approaches and statistical analyses

Four target trial emulations to answer our research question were performed (**Table 1**; **Fig. 1**). First, hypothetical target trials were formulated. As the different approaches emulated different analogues of randomization, three hypothetical target trials were specified, which differed in the way treatment was assigned. **Table 1** describes each element of the hypothetical trials and the corresponding emulations. In all approaches, loss to follow-up date was defined as the completion date of the participant's most recent scheduled questionnaire, as information on vaccinations and infection might be incomplete thereafter. A sensitivity analysis was performed, in which loss to follow-up date was defined as the first date a scheduled questionnaire was not completed, i.e. date of protocol deviation. Additionally, for the first approach we performed a sensitivity analysis in which we included the four most common medical risk conditions (diabetes mellitus, lung disease or asthma, cardiovascular disease and immune deficiency) and "other conditions" each as a 0/1 covariate in the model. Covariates were time-varying and participants could have multiple conditions.

All analyses were performed with R software version 4.4.3, using packages ipw (version 1.2.1) [14], survival (version 3.7.0), and boot (version 1.3.31).

Table 1 Target trial protocol for estimating vaccine effectiveness of XBB.1.5 booster vaccination against SARS-CoV-2 infection

Protocol component	Hypothetical target trial	Emulated target trial (single time zero with covariate adjustment)	Emulated target trial (single time zero with inverse probability weighting)	Emulated target trial (multiple time zeros with covariate matching)	Emulated target trial (multiple time zeros with inverse probability weighting)
Eligibility criteria	- Age 18-85 years - Eligible for XBB.1.5 booster vaccination at baseline (HCW, 60+, medical risk; no infection in 90 days prior) - Understanding Dutch	- Age 18-85 years at time zero - Eligible for XBB.1.5 booster vaccination (HCW, 60+, medical risk; no infection in 90 days prior) - Understanding Dutch	- Age 18-85 years at time zero - Eligible for XBB.1.5 booster vaccination (HCW, 60+, medical risk; no infection in 90 days prior) - Understanding Dutch	- Age 18-85 years at time zero - Eligible for XBB.1.5 booster vaccination (HCW, 60+, medical risk, no COVID-19 vaccine dose received since 2 October 2023, no infection in 90 days prior) - Understanding Dutch	- Age 18-85 years at time zero - Eligible for XBB.1.5 booster vaccination (HCW, 60+, medical risk, no COVID-19 vaccine dose received since 2 October 2023, no infection in 90 days prior) - Understanding Dutch
Treatment strategies	1. Administer XBB.1.5 booster vaccine 2. Do not administer XBB.1.5 booster vaccine	1. Administer XBB.1.5 booster vaccine 2. Do not administer XBB.1.5 booster vaccine	1. Administer XBB.1.5 booster vaccine 2. Do not administer XBB.1.5 booster vaccine	1. Administer XBB.1.5 booster vaccine 2. Do not administer XBB.1.5 booster vaccine	1. Administer XBB.1.5 booster vaccine 2. Do not administer XBB.1.5 booster vaccine
Treatment assignment	Emulation 1: all-at-once enrolment and randomization	Adjustment for potential time-fixed and time-varying	Adjustment for potential time-fixed and time-varying	1:1 matching on calendar time (by using sequential trials defined	Matching on calendar time (by using sequential trials defined by

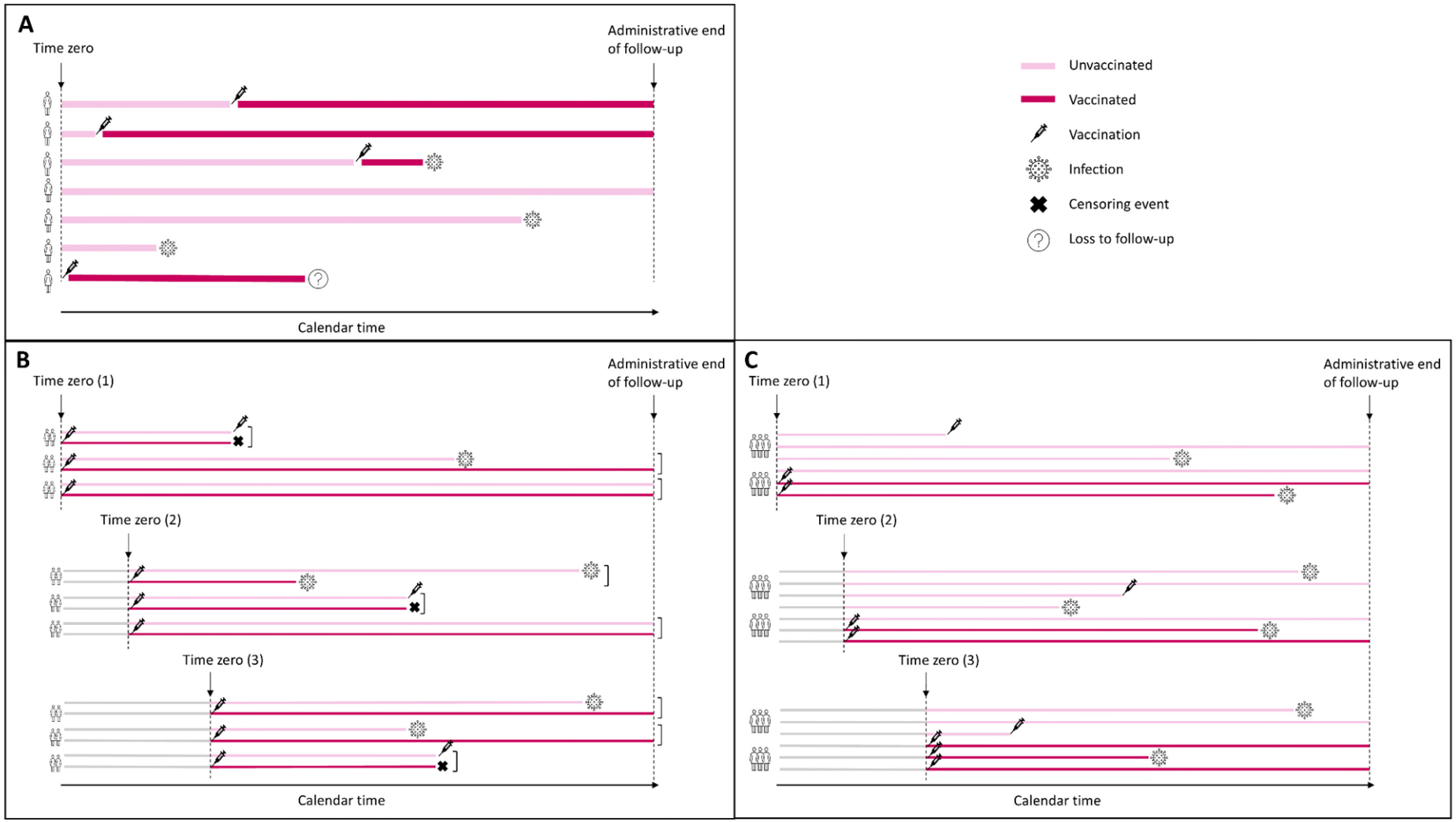
	Emulation 2: all-at-once enrollment and randomization Emulation 3: sequential enrolment (by day) and stratified sequential randomization, i.e. eligible participants are randomly assigned to a vaccination strategy on each day as well as within strata defined by covariates. Emulation 4: sequential enrolment (by week) and (non-stratified) sequential randomization, i.e. eligible participants are randomly assigned to a vaccination strategy within each week	confounders by regression analysis.	confounders by inverse probability weighting	by calendar date) and other potential confounders at time zero.	calendar week) + adjustment for potential confounders by inverse probability weighting at time zero.
Follow-up	Time zero is when eligibility criteria for inclusion are met,	Time zero is the start of the vaccination roll-out on 2	Time zero is the start of the vaccination roll-out on 2	Time zero is date of vaccination or equivalent index date of the	Time zero is date of vaccination or equivalent index week of any

	treatment is assigned, and follow-up begins. Follow-up is from time zero to outcome, loss-to-follow up, death, or administrative end of follow-up, whichever occurred first.	October 2023. Follow-up time starts at time zero as unvaccinated person-time and switches to vaccinated person-time at the day of vaccination. Follow-up is until outcome, loss to follow-up (incl. due to death) or administrative end of follow-up, whichever occurred first.	October 2023. Follow-up time starts at time zero as unvaccinated person-time and switches to vaccinated person-time at the day of vaccination. Follow-up is until outcome, loss to follow-up (incl. due to death) or administrative end of follow-up, whichever occurred first.	matched control who did not receive vaccination on or before that date. Follow-up is from time zero to outcome, loss to follow-up (incl. due to death), receipt of a vaccine-dose by an individual in the unvaccinated group (with concurrent censoring of the vaccinated member of the pair), or administrative end of follow-up, whichever occurred first.	control who did not receive vaccination on or before that date. Follow-up is from time zero to outcome, loss to follow-up (incl. due to death) , receipt of a vaccine-dose by an individual in the unvaccinated group, or administrative end of follow-up, whichever occurred first.
Outcome	SARS-CoV-2 infection between treatment allocation and end of study	SARS-CoV-2 infection (self-reported or serology-based) between 2 October 2023 and 2 April 2024	SARS-CoV-2 infection (self-reported or serology-based) between 2 October 2023 and 2 April 2024	SARS-CoV-2 infection (self-reported or serology-based) between index date and 2 April 2024	SARS-CoV-2 infection (self-reported or serology-based) between index week and 2 April 2024
Causal contrast of interest	Per-protocol, individuals in the control arm were censored upon receiving vaccination	Per-protocol	Per-protocol	Per-protocol	Per-protocol
Statistical analysis	Cumulative incidence (risk) curves of SARS-COV-2	Cox regression model with calendar time as underlying	Cox regression model with time-varying exposure,	Cumulative incidence (risk) curves using the Kaplan-Meier	Cox regression model stratified by trial, adjusting for covariates

	infection using the Kaplan-Meier estimator; vaccine effectiveness = 100% * (1 – risk ratio (RR))	time scale and time-varying exposure, with regression adjustment for (time-varying) covariates; vaccine effectiveness = 100% * (1 – hazard ratio (HR))	weighted by time-varying IPTWs and IPCWs; vaccine effectiveness = 100% * (1 – hazard ratio (HR)) and robust standard error estimate	estimator; vaccine effectiveness = 100% *(1 – risk ratio (RR)), based on the median of 50 repetitions of the matching procedure, and bootstrapping the entire analysis to compute 95%CI	using IPTW and IPCW; vaccine effectiveness = 100% * (1 – hazard ratio (HR)) and bootstrapping to compute 95%CI

HCW = healthcare worker; IPTW = inverse probability treatment weight(ing); IPCW = inverse probability censoring weight(ing); RR = risk ratio; HR = hazard ratio

Fig. 1 Visualization of the target trial emulation approaches



A) Single time zero approaches (approach 1 and 2); **B)** Multiple time zeros with covariate matching (approach 3); **C)** Multiple time zeros with inverse probability weighting (approach 4)

1. Single time zero with regression adjustment

This first approach is a time-dependent Cox model, which is widely used for estimating VE in cohort studies with time-to-event data, but not usually considered part of the TTE canon [15, 16]. Probably this approach is hardly used within the TTE framework because it is known to produce biased estimates in the presence of time-varying confounders that are themselves affected by the treatment [17-19]. However, treatment-confounder feedback by the measured time-varying confounders in our model seems unlikely.

The first eligible time was chosen as time zero. Participants were eligible for XBB.1.5 booster vaccination at the start of the vaccination roll-out on 2 October 2023 or three months after their most recent prior SARS-CoV-2 infection, whichever occurred last. Follow-up started at time zero. Treatment assignment was not aligned at time zero, because participants could receive a vaccination at any time between time zero and the end of the vaccination campaign. To account for this, vaccination status was coded as a time-varying variable that transitioned from 'unvaccinated' to 'vaccinated' seven days after administration of the XBB.1.5 booster vaccination and remained 'vaccinated' thereafter. The seven days after vaccination administration were excluded from the analysis. Follow-up ended on the date of the first SARS-CoV-2 infection, loss to follow-up or the end of the study period on 2 April 2024, whichever came first.

A Cox proportional hazards model with calendar time as the underlying time scale was used to estimate VE. The estimate was adjusted for potential confounding by adding age group, sex, medical risk condition, educational level, and time since prior infection as covariables to the Cox regression model. Age group, medical risk condition and time since prior infection were time-varying covariates. VE was calculated as 100% × (1 − hazard ratio). To evaluate VE by time since vaccination, the exposure was categorized into six-week periods with 'unvaccinated' as the reference category.

2. Single time zero with inverse probability weighting

Robins' generalized methods (G-methods) are a class of methods that, unlike stratification or standard regression (e.g. the time-dependent Cox model used in our first approach), appropriately adjusts for confounding when treatment-confounder feedback exists[19]. Among available G-methods, models using time-varying inverse probability weights (IPWs), also known as marginal structural models, are most commonly used for our type of data and causal question [18].

Eligibility criteria, follow-up time and the exposure were defined in the same manner as in the first approach. However, we used a Cox proportional hazards model with inverse probability of treatment weighting (IPTW) to adjust for confounding and inverse probability of censoring weighting (IPCW) to adjust for informative censoring due to non-random loss to follow-up [14, 20, 21]. The final weights were obtained by multiplying the stabilized time-varying IPTWs and IPCWs. The weighting procedures are described in detail in the **Supplementary file, Statistical analyses**.

Finally, a Cox proportional hazards model, weighted by the time-varying stabilized weights and additionally adjusted for the time-fixed confounders, was fit. This adjustment was required because stabilized weights do not adjust for the confounders that were included in the numerator model [1, 20]. To account for person-level clustering induced by weighting, a robust standard error estimate was obtained. VE by time since vaccination was evaluated by categorizing the exposure into six-week periods with 'unvaccinated' as the reference category.

3. Multiple time zeros with covariate matching

In the third approach, we used every eligible time as time zero, i.e. multiple time zeros. For each calendar day of the vaccination period, persons who met the eligibility criteria on that day, and who had not been vaccinated before that day, were identified and classified as either having or not having received XBB.1.5 booster vaccination on that day. This way, a sequence of 'trials' was

created, with a new trial starting on each day. Each person who received a vaccination was matched exactly to a randomly selected control (with replacement) on age group, sex, medical risk condition, educational level, and time since prior infection. Eligible individuals could be selected as unvaccinated controls repeatedly up to the day before they were vaccinated. For each matched pair, follow-up started on the day of vaccine administration of the vaccinated member of the pair and ended at the date of the first reported SARS-CoV-2 infection, the vaccination date of the control, loss to follow-up or the end of the study period, whichever came first.

Cumulative incidence curves of self-reported positive SARS-CoV-2 test were constructed using the Kaplan-Meier estimator. As confounding due to observed baseline covariates was addressed by matching, additional covariate adjustment was not required. Both individuals of a matched pair had to be still at risk by day 8 of follow-up. The risk of SARS-CoV-2 infection more than 7 days after vaccination was compared with the risk among their matched controls. As matching is random, it introduces variability in the effect estimates. Therefore, to obtain the VE point estimate, matching was performed 50 times, with random reordering of the data preceding each matching run. VE was calculated as 100% × (1 – risk ratio), using the median of the 50 estimates. 95% confidence intervals (CIs) were computed using bootstrapping (1000 iterations), to account for sequential trials not being independent. Bootstrap samples were obtained by randomly sampling individuals with replacement. Matching and VE estimation were performed within each bootstrap iteration using all data points for each sampled individual. To estimate period-specific VE from the KM curve for each six-week period since vaccination, we included only pairs that were not censored before the start of the period and censored them at the end of the period.

4. Multiple time zeros with inverse probability weighting

Similar to the third approach, in the fourth approach all eligible times were used as time zero. When selecting trials per day, estimation of the IPCWs resulted in non-positivity in some of the groups due

to the absence of censoring. Therefore, we created trials per week, rather than per day. In each week of the study period, we identified persons who had not been vaccinated before that week (index week), who met the eligibility criteria, and who still met the eligibility criteria 7 days later at the start of follow-up. All participants who did not receive XBB.1.5 booster vaccination in that week were classified as unvaccinated. All participants who did receive XBB.1.5 booster vaccination sometime during that week were classified as vaccinated. Eligible individuals were enrolled as unvaccinated in each trial up to the week before their vaccination, but once they received vaccination they could not enroll in subsequent trials. We excluded weeks in which <20 individuals received a XBB.1.5 booster vaccination or <20 individuals remained unvaccinated to avoid model separation when estimating the probability of censoring. Follow-up started one week after each index week (i.e. 7 to 13 days after an individual's vaccination date) and was continued until the week of the first reported SARS-CoV-2 infection, loss to follow-up or the end of the study period, whichever came first. Unvaccinated controls were censored at the end of the week of their vaccination. IPW was used to adjust for confounding (IPTW) and informative censoring due to loss to follow-up ($IPCW_{LTFU}$) and due to protocol deviation, i.e. vaccination of unvaccinated controls during follow-up ($IPCW_{PD}$) [11]. The computation of the IPWs is described in the **Supplementary file, Statistical analyses**. The final weights were obtained by multiplying the stabilized IPTWs, $IPTWs_{PD}$ and $IPCWs_{LTFU}$.

Finally, weighted Cox models stratified by trial were fit. VE was calculated as 100% × (1 − hazard ratio). The weighted model was adjusted for the time-fixed confounders that were included in the numerator model of the IPCWs. The 95% CIs were computed using bootstrapping (1000 iterations). To evaluate VE by time since vaccination, the exposure was categorized into six-week periods with 'unvaccinated' as the reference category.

RESULTS

1. Single time zero with regression adjustment

In total, 24,288 VASCO participants were eligible for XBB.1.5 booster vaccination and were included in the analysis. Of those, 15,300 (63%) received vaccination during the study period (**Table 2**). Participants who received vaccination were older, more often male, more often had a high education level, more often had a medical risk condition, and less often had experienced a prior infection. During 265,660 vaccinated and 202,961 unvaccinated person-weeks, 2,900 and 3,318 infections occurred, respectively. Risk of infection was higher among unvaccinated participants (**Supplementary file, Fig. S1**). Furthermore, incidence was highest among those without prior infection and lowest among those with a prior infection in the past year (**Supplementary file, Fig. S2**). Overall VE against infection was 30% (95% CI 26-34) (**Fig. 2**). VE decreased as time since vaccination increased, being 35% (30-39), 24% (18-30) and 8% (-9-23) at 1-6, 7-12 and 13-18 weeks post-vaccination, respectively (**Fig. 2; Supplementary file, Table S1**).

Table 2 Characteristics of participants included in single time zero with regression adjustment analysis and multiple time zeros with covariate matching analysis, the Netherlands, 2 October 2023 – 2 April 2024

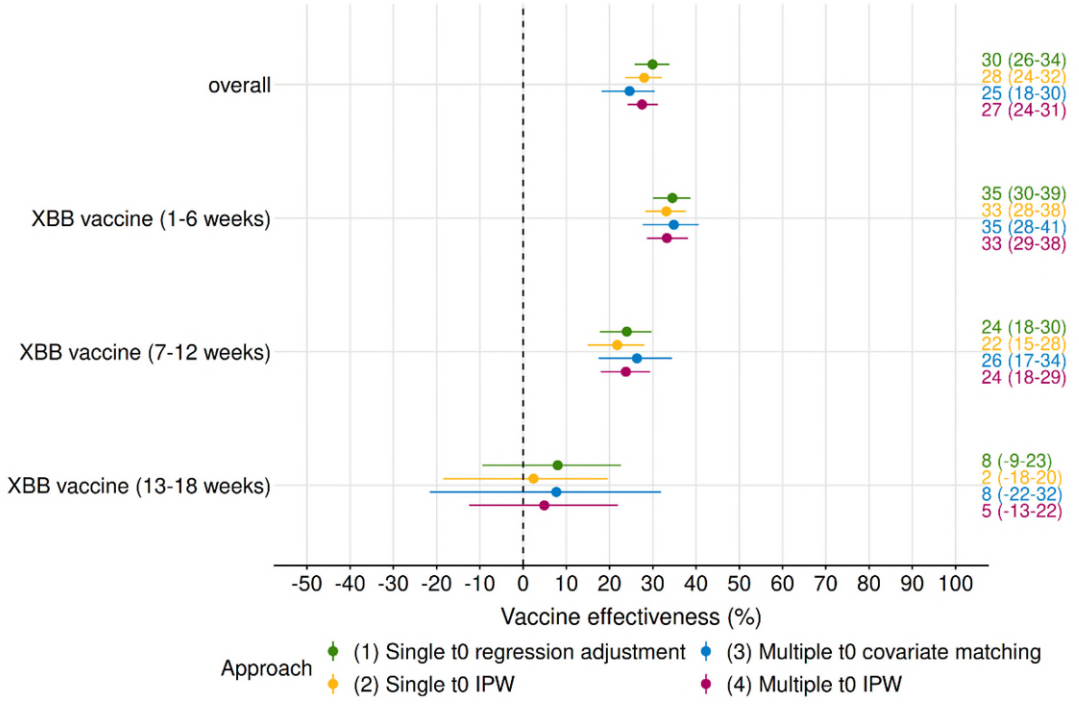
	Single time zero with regression adjustment			**Multiple time zeros with covariate matching[b]**		
	Total	**No XBB.1.5 booster vaccination[a]**	**Yes XBB.1.5 booster vaccination[a]**	**Total**	**No XBB.1.5 booster vaccination**	**Yes XBB.1.5 booster vaccination**
	n = 24288	n = 8988	n = 15300	n = 25808	n = 12904	n = 12904
Age group						
18-39	1083 (4.5%)	822 (9.1%)	261 (1.7%)	494 (1.9%)	247 (1.9%)	247 (1.9%)
40-59	4094 (16.9%)	2888 (32.1%)	1206 (7.9%)	2302 (8.9%)	1151 (8.9%)	1151 (8.9%)
60-69	13457 (55.4%)	4137 (46%)	9320 (60.9%)	16256 (63%)	8128 (63%)	8128 (63%)
70-85	5654 (23.3%)	1141 (12.7%)	4513 (29.5%)	6756 (26.2%)	3378 (26.2%)	3378 (26.2%)
Sex						
Female	15008 (61.8%)	6293 (70%)	8715 (57%)	14842 (57.5%)	7421 (57.5%)	7421 (57.5%)
Male	9276 (38.2%)	2693 (30%)	6583 (43%)	10966 (42.5%)	5483 (42.5%)	5483 (42.5%)
Other	4 (0%)	2 (0%)	2 (0%)	0 (0%)	0 (0%)	0 (0%)

Education level						
High	13418 (55.2%)	4677 (52%)	8741 (57.1%)	14544 (56.4%)	7272 (56.4%)	7272 (56.4%)
Intermediate	6835 (28.1%)	2841 (31.6%)	3994 (26.1%)	6942 (26.9%)	3471 (26.9%)	3471 (26.9%)
Low	3878 (16%)	1415 (15.7%)	2463 (16.1%)	4158 (16.1%)	2079 (16.1%)	2079 (16.1%)
Other	157 (0.6%)	55 (0.6%)	102 (0.7%)	164 (0.6%)	82 (0.6%)	82 (0.6%)
Medical risk condition						
Yes	11384 (46.9%)	4099 (45.6%)	7285 (47.6%)	11912 (46.2%)	5956 (46.2%)	5956 (46.2%)
Time since prior infection						
<1 year ago	3033 (12.5%)	1553 (17.3%)	1480 (9.7%)	9004 (34.9%)	4502 (34.9%)	4502 (34.9%)
>1 year ago	17857 (73.5%)	6394 (71.1%)	11463 (74.9%)	12922 (50.1%)	6461 (50.1%)	6461 (50.1%)
No prior infection	3398 (14%)	1041 (11.6%)	2357 (15.4%)	3882 (15%)	1941 (15%)	1941 (15%)

[a] For the purpose of this table, vaccination status and all covariates were determined at the end of follow-up

[b] Characteristics of the matched population of which the VE estimate was closest to the point estimate.

Fig. 2 Vaccine effectiveness estimates overall and by time since vaccination, by emulation approach



IPW = inverse probability weighting

2. Single time zero with inverse probability weighting

In the second approach, the same participants were included as in approach 1. None of the estimated stabilized IPWs was >10, thus truncation to 10 for extreme IPWs was not applied. Assessment of the balance of the time-varying variables between the vaccinated and unvaccinated population is shown in the **Supplementary file, Fig. S3)**. Overall VE against infection was 28% (24-32) (**Fig. 2**). VE decreased as time since vaccination increased, with 33% (28-38), 22% (15-28) and 2% (-18-20) at 1-6, 7-12 and 13-18 weeks post-vaccination, respectively (**Fig. 2; Supplementary file, Table S1**).

3. Multiple time zeros with covariate matching

In the matched study population that yielded the VE estimate closest to the median of the 50 repetitions, 2,664 matched pairs were excluded due to the requirement that both individuals in a matched pair remain at risk through day 8. On 95 dates at least one person was vaccinated and could be matched to a (yet) unvaccinated individual. The number of matched pairs ranged between 1 and 350 per trial (**Supplementary file, Fig. S4**). In total, 12,904 vaccinated participants (out of 15,300) were matched to unvaccinated participants (**Table 2**), in whom 1,409 and 1,844 infections occurred, respectively (**Supplementary file, Fig. S5**). Most characteristics of included participants were comparable to characteristics of vaccinated individuals in the entire study population (as included in approach 1). Matched vaccinated individuals were less often in the oldest age group (≥70 years), possibly because vaccination coverage in this age group was high and few unvaccinated were available for matching. Overall VE against infection was 25% (18-30) (**Fig. 2**). The interquartile range of the VE point estimate based on 50 repetitions was 23-26. VE decreased as time since vaccination

increased with 35% (28-41), 26% (17-34) and 8% (-22-32) at 1-6, 7-12 and 13-18 weeks post-vaccination, respectively (**Fig. 2; Supplementary file, Table S1**).

4. Multiple time zeros with inverse probability weighting

A trial was created, i.e. a vaccinated and a (still) unvaccinated group were defined and follow-up of these groups started, in 12 different calendar weeks. Eight weeks were excluded because of <20 individuals in the vaccinated and/or unvaccinated group; the week of Christmas and seven weeks after the official end of the vaccination campaign. The number of participants ranged between 5,722 and 20,871 per trial. In total, 141,638 non-unique individuals were included. After weighting, the vaccinated and unvaccinated population were comparable (**Supplementary file, Fig. S6**). Truncation of large IPWs was only required for <0.01% of the estimated IPWs. In total, 2,744 and 19,886 infections occurred in vaccinated and unvaccinated participants (**Supplementary file, Fig. S7**). Overall VE against infection was 27% (24-31) (**Fig. 2**). VE decreased as time since vaccination increased, with 33% (29-38), 24% (18-29) and 5% (-13-22) at 1-6, 7-12 and 13-18 weeks post-vaccination, respectively (**Fig. 2; Supplementary file, Table S1**).

The sensitivity analysis in which loss to follow-up date was defined as the first day that a scheduled questionnaire was not completed resulted in negligible differences in the estimates of the four approaches (**Supplementary file, Fig. S8**). The second sensitivity analysis of the first approach, in which we adjusted for medical risk condition more finely, also resulted in negligible differences in the estimates (**Supplementary file, Fig. S9**).

DISCUSSION

In this study we used four TTE approaches to estimate VE of the XBB.1.5 booster vaccination against SARS-CoV-2 infection. The four approaches yielded very similar overall VE estimates ranging

between 25 and 30%, with considerably overlapping 95% confidence intervals. Likewise, the estimates of VE by time since vaccinations were similar between the approaches.

VE estimated by this study appears somewhat lower than generally found in the literature, possibly because other studies assessed more severe outcomes, i.e. symptomatic or medically attended infections. A more detailed interpretation of the literature can be found elsewhere [12, 22]. In line with the aim of our manuscript to compare methodological approaches, we focus on this comparison in our discussion.

To start with, the estimand of the multiple time zeros with covariate matching approach (approach 3) differs from the other three approaches in two ways. Firstly, approach 3 estimates the average treatment effect on the treated (ATT), or, strictly speaking, the average treatment effect in the overlap population (ATO), as opposed to the overall average treatment effect (ATE). The ATE estimates the effect of treatment across the entire population regardless of whether individuals actually received treatment. It is the difference between the expected outcome if all individuals were treated and the expected outcome if none were treated. The ATT focuses on the effect only within those individuals that actually received treatment. This is a consequence of the matching in approach 3. As some vaccinated individuals who could not be matched were removed, actually approach 3 estimates the ATO: the average treatment in the overlap/equipoise population, i.e. the population with comparable chances of being (un)treated based on the matching variables [23]. Differences between the ATE and ATT or ATO arise when the treatment effect varies between population subgroups (i.e., interactions between the treatment and individual characteristics) and the treated and untreated differ in these characteristics. Our data indeed showed differences in characteristics of vaccinated (treated) and unvaccinated (untreated) individuals, therefore at least the second requirement holds true. Secondly, approach 3 estimates a marginal effect, whereas the other approaches estimate a conditional effect. Conditional effects control for the values of other variables, making them context-dependent. Marginal effects summarize the average effect of a

variable across the entire distribution of other variables of the model [24]. For collapsible measures of effect, like the risk ratio estimated in approach 3, the marginal and conditional effects are the same in the absence of interactions between the exposure and covariates. For non-collapsible measures of effect, like the hazard ratio estimated in approaches 1, 2 and 4, the conditional effect differs from the marginal effect if the covariates that are conditioned on are predictive of the outcome, even if there is no association with the exposure (i.e. confounding) or effect modification [24]. Because the estimand of the multiple time zeros with covariate matching approach differs from the other approaches in these ways, the effect estimates are not directly comparable and, although this is not evident in our results, their numeric value may differ.

Furthermore, the analysis of VE by time since vaccination using the multiple time zeros with covariate matching approach differs from the other three approaches. When estimating period-specific hazard ratios (HRs), only participants who remain event-free (and do not drop out) until a certain time are included in the subsequent period. Individuals who are at increased risk of infection, e.g. due to weaker immunity or greater exposure, are likely to get infected earlier, thus leaving a lower-risk population at longer times since vaccination. Infections are likely to occur at a faster rate in the unvaccinated group, as this group is not protected by vaccination, leading to more rapid depletion of susceptibles than in the vaccinated group. This differential depletion of susceptibles can make period-specific VE appear artificially low long after vaccination [7, 25]. In contrast, in our approach using multiple time zeros with covariate matching, both members of each matched pair were required to stay event-free (and not drop out) until the start of the time interval since vaccination. As a result, vaccinated and unvaccinated individuals may be more comparable with respect to susceptibility of infection given the measured confounders that were used for matching. Therefore, this approach may be less affected by spurious waning caused by differential depletion of susceptibles. In our analyses, we did not observe numerical differences in the period-specific estimates between the approaches, although CIs were rather wide. This suggests that lower VE with increasing time since vaccination either reflects true waning or that the matching variables were

insufficient to ensure similar infection risk in the vaccinated and unvaccinated groups. Any effect on the estimated VE may also have been obscured by the aforementioned differences in estimands. Nonetheless, if substantial differential depletion of susceptibles is expected, multiple time zeros with covariate matching may be the preferred approach to investigate VE by time since vaccination.

The methods used have varying assumptions and limitations. First, Kaplan-Meier (approach 3) is a non-parametric method with no assumptions about the distribution of the unobserved failure times due to administrative censoring. In contrast, the semi-parametric Cox regression model (used in approaches 1, 2 and 4), while not assuming a particular distribution for failure times or hazards either, imposes a priori restrictions on the relation between the baseline hazard and the hazard under other combinations of covariate values [26]. Second, the approach using a single time zero with Cox regression is biased if a time-varying covariate that is affected by vaccination status is adjusted for in the regression model, so this approach is methodologically inappropriate in the presence of treatment-confounder feedback [17-19]. Studies that measured such covariates and want to appropriately adjust for them should avoid this approach. Third, using multiple time zeros with covariate matching requires bootstrapping to estimate CIs to account for variation introduced by matching and correct for false precision due to non-independence of the data. This approach also results in a smaller number of participants and outcomes due to matching in case not every vaccinated individual can be matched to an unvaccinated individual and due to censoring of the entire pair when the unvaccinated received vaccination. This may result in wider CIs, particularly with increasing time since vaccination. By matching, this method also eliminates the possibility of studying the effect of individual confounders in sensitivity analyses [27]. Finally, the multiple time zeros with IPW approach is computationally intensive and requires a lot of data. In our study, we created trials per week rather than per day because of non-positivity, but we expect limited effect of this on our results. It was impossible to estimate the $IPCW_{LTFU}$ by trial because this type of censoring was too rare, so we estimated the $IPCW_{LTFU}$ in the dataset including all trials. This assumes that the

associations between the covariates and the probability of censoring due to loss to follow-up are the same across trials.

The TTE framework is useful to systematically think through every step of the design and analysis of an observational study aiming to answer a causal question. This way, it helps researchers to recognize, appropriately deal with, and communicate about methodological pitfalls and potential biases inherent to the use of observational data for causal inference [9, 27, 28]. Nonetheless, the TTE framework has several caveats. First, the benefit of the framework depends on how rigorously it is applied and this needs to be fully reported for readers to understand and assess. One systematic review reported that only 58% of studies explicitly aiming to emulate a target trial completely report how the target trial was emulated [9, 29]. Second, applying the TTE framework can still lead to multiple 'as correct as possible' study designs that may yield comparable estimates, as was the case in our study. On the other hand, if the estimates do differ between approaches, it may be difficult to uncover which one is (most) correct. Third, studies designed using the TTE framework may still be flawed, because TTE does not remove the limitations of the data, such as use of self-reported data, or unmeasured confounding [27]. Both of these limitations apply to the data used for this study, as discussed in more detail in Huiberts et al. [13, 22]. In addition, in this study, we had to assume non-interference [8] and could only assess the direct effect of vaccination. We also did not have sufficient participants to estimate VE by subgroup eligible for vaccination, i.e. among persons aged ≥60 and among younger clinically vulnerable individuals. Fourth, (too) strict adherence to the TTE framework can be contentious. Overzealous application/interpretation of the TTE framework would suggest that studies should have a prospective cohort design in order to mimic an RCT, but case-control studies are valuable too and can indeed also be designed within the TTE framework [30]. For COVID-19 VE specifically, test-negative case-control designs have been advantageous to reduce bias introduced by health-seeking behavior. It is also debatable whether the single time zero approaches can be considered properly emulated target trials within the TTE framework, because treatment assignment is not aligned at time zero. However, these approaches handle this by allowing

vaccination status to be time-varying, so it can be argued that the optimal analytical approach does not necessarily need to align eligibility, treatment assignment and the start of follow-up to obtain an unbiased estimate. Indeed, the similarity of our estimates suggests that these approaches do not provide inherently less reliable effect estimates. In short, TTE is neither a condition for nor a guarantee of unbiased causal inference, but it helps to avoid methodological pitfalls and biases.

CONCLUSIONS

In this study, the hypothetical target trial to estimate the VE of the XBB.1.5 booster vaccination was emulated using four different design and analysis approaches. Although we cannot know the true VE, the VE estimates were similar across the four approaches. To the extent that the four approaches have different assumptions and estimands, we can conclude that the VE estimates in this study are not sensitive to these differences. This study highlights that observational data can be analyzed in multiple valid ways to answer a causal research question. The choice of approach should depend on the estimand of interest (e.g. ATT vs. ATE, marginal vs. conditional effect), the plausibility of the assumptions of various approaches (e.g. proportional hazards, no treatment-confounder feedback) and practical and technical feasibility (e.g. computational power, data quantity).

LIST OF ABBREVIATIONS

ATE = average treatment effect

ATO = average treatment effect in the overlap population

ATT = average treatment effect on the treated

COVID-19 = Coronavirus Disease 2019

G-methods = generalized methods

HR = hazard ratio

IPCW = inverse probability of censoring weight(ing)

IPTW = inverse probability of treatment weight(ing)

IPW = inverse probability weight(ing)

LTFU = loss to follow-up

PD = protocol deviation

SARS-CoV-2 = Severe Acute Respiratory Syndrome Coronavirus 2

TTE = target trial emulation

VASCO = VAccine Study COvid-19

VE = vaccine effectiveness

DECLARATIONS

Ethics approval and consent to participate

Written informed consent was obtained from all participants prior to enrolment into the study. The VASCO study is conducted in accordance with the principles of the Declaration of Helsinki and the study protocol was approved by the Medical Ethics Committee of the *Stichting Beoordeling Ethiek Biomedisch Onderzoek*, Assen, the Netherlands.

Consent for publication

Not applicable.

Availability of data and materials

Anonymized data reported from this study can be obtained from the corresponding author upon request. The dataset may include individual data and a data dictionary will be provided. Data requests should include a proposal for the planned analyses. Data transfer will require a signed data sharing agreement. Code for data processing, statistical analysis, figures, and tables can be found at GitHub (https://github.com/b-smagge/TTE_COVID_VE).

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

All authors have read and approved the final manuscript. AH, BS, HvW, BdG and MK conceptualized and designed the study. AH and BS performed data cleaning and data analysis. AH and BS drafted the manuscript. HvW, BdG, SM, HdM, SH, DG, JvdW, SvdH and MK critically reviewed the paper.

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