



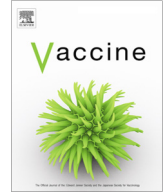
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Comparison of test-negative and syndrome-negative controls in SARS-CoV-2 vaccine effectiveness evaluations for preventing COVID-19 hospitalizations in the United States

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ABSTRACT

Background: Test-negative design (TND) studies have produced validated estimates of vaccine effectiveness (VE) for influenza vaccine studies. However, syndrome-negative controls have been proposed for differentiating bias and true estimates in VE evaluations for COVID-19. To understand the use of alternative control groups, we compared characteristics and VE estimates of syndrome-negative and test-negative VE controls.

Methods: Adults hospitalized at 21 medical centers in 18 states March 11–August 31, 2021 were eligible for analysis. Case patients had symptomatic acute respiratory infection (ARI) and tested positive for SARS-CoV-2. Control groups were test-negative patients with ARI but negative SARS-CoV-2 testing, and syndrome-negative controls were without ARI and negative SARS-CoV-2 testing. Chi square and Wilcoxon rank sum tests were used to detect differences in baseline characteristics. VE against COVID-19 hospitalization was calculated using logistic regression comparing adjusted odds of prior mRNA vaccination between cases hospitalized with COVID-19 and each control group.

Results: 5811 adults (2726 cases, 1696 test-negative controls, and 1389 syndrome-negative controls) were included. Control groups differed across characteristics including age, race/ethnicity, employment, previous hospitalizations, medical conditions, and immunosuppression. However, control-group-specific VE estimates were very similar. Among immunocompetent patients aged 18–64 years, VE was 93 % (95 % CI: 90–94) using syndrome-negative controls and 91 % (95 % CI: 88–93) using test-negative controls.

Conclusions: Despite demographic and clinical differences between control groups, the use of either control group produced similar VE estimates across age groups and immunosuppression status. These findings support the use of test-negative controls and increase confidence in COVID-19 VE estimates produced by test-negative design studies.

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1. Background

Since the first Emergency Use Approval (EUA) for a COVID-19 vaccine from the World Health Organization (WHO) in December 2020, there has been ongoing investigation of performance of these vaccines in real-world settings [1]. Post-authorization evaluations of vaccine performance help to determine vaccine effectiveness (VE) in the context of clinical and implementation factors not fully assessed by licensure clinical trials such as underlying medical conditions, duration of protection, comparison of vaccine products and types, protection against emerging variants, and diverse clinical outcomes [2–5]. Vaccine effectiveness studies also address evidence gaps on protection against illness progression that are critical for evaluating diseases such as COVID-19 with an increased risk of severe outcomes in select populations [6–8]. In consideration of the unique epidemiology of COVID-19, the WHO recommends the test-negative design (TND) for use in COVID-19 VE evaluations; however, methodological challenges remain with evaluation of COVID-19 [9].

TND is an established tool for evaluating vaccine effectiveness in influenza and rotavirus [5,10–11]. TND combines features of both prospective cohort and case control studies as patients with a clinical syndrome are prospectively enrolled prior to knowledge of disease status, and laboratory testing is used to perform post-hoc classification of patients as cases and controls [11]. Control-patients who present with the clinical syndrome of interest (e.g., acute respiratory illness [ARI]) but are not infected with the vaccine-preventable pathogen of interest (e.g., influenza virus) are classified as “syndrome-positive, test-negative controls” (hereafter “test-negative controls”) [12]. In contrast to TND, traditional case-control studies include “disease-free” control-patients without the clinical syndrome of interest (hereafter “syndrome-negative controls”). TND offers two main advantages over traditional case-control studies: (1) simplified enrollment of control-patients who are captured during the case identification process; and (2) comparable healthcare seeking behavior between case- and control-patients as they have the same clinical syndrome. Due to these logistical and methodologic advantages, TND has been

widely employed as the *de facto* standard for evaluating COVID-19 vaccine effectiveness during the pandemic [9,13]. Evaluations of control groups between TND and traditional case-control designs for influenza and rotavirus VE studies show good comparability [14–17]. However, while theoretical comparisons between the study designs have been detailed in the literature, empirical evidence on control group comparability does not yet exist for COVID-19 VE evaluations [18].

The primary purpose of control groups in VE studies is to provide vaccine coverage estimates among people without the infection of interest from the same population as case-patients. For COVID-19 VE studies, controls could be patients with an ARI who test-negative for SARS-CoV-2 (test-negative controls) or patients without an ARI (syndrome-negative controls). An advantage of using a test-negative control group is that case- and control-patients exhibit the same healthcare seeking behavior for an ARI, thereby minimizing potential selection bias stemming from differential care-seeking across vaccination status [19]. Conversely, while the use of test-negative controls can minimize selection bias, collider bias may be introduced in TND studies when both health-seeking behavior and SARS-CoV-2 infections may lead to COVID-19 testing.

One advantage of using a syndrome-negative control group is that it minimizes misclassification of case-control status. Since control-patients do not overlap in symptoms with case-patients (ARI or COVID-like illness), false-negative test results are less likely to occur. This reduction can be critical during times of higher prevalence of SARS-CoV-2 in the population, when imperfect test sensitivity can lead to an increase in false negatives [20]. Additionally, high COVID-19 prevalence itself may limit non-COVID ARIs and thus create challenges with enrolling test-negative controls. Conversely, while false positive test results are less common in COVID-19 than false negatives, this type of misclassification is a greater concern for bias in VE particularly in TND studies as true positives may be over-represented in a test-negative control group [4,20–22]. Thus, use of syndrome-negative controls may be more desirable for reducing misclassification bias.

The Centers for Disease Control and Prevention (CDC) in collaboration with the Influenza and Other Viruses in the Acutely Ill (IVY) Network has conducted a series of observational studies evaluating the effectiveness of COVID-19 vaccines against COVID-19 hospitalization among US adults [23]. Due to concerns about potential biases from different control groups and lack of evidence supporting the use of a particular type of control in COVID-19 VE studies, the IVY network enrolled both test-negative controls and syndrome-negative controls. In this paper, we examine these two control groups with regard to baseline characteristics, vaccination coverage, and VE estimates generated from analyzing each control group separately.

2. Methods

2.1. Setting and participants

The IVY public health surveillance network has been enrolling adults hospitalized with COVID-19 and concurrent controls throughout the pandemic within a 21-site consortium in the United States. This study included patients hospitalized between March 11, 2021 and August 31, 2021, the period in which both test-negative and syndrome-negative controls were enrolled.

Three groups of hospitalized adults (age ≥ 18 years) were enrolled based on clinical signs and symptoms and clinically available SARS-CoV-2 test results: COVID-19 cases, test-negative controls and syndrome-negative controls. Cases were defined as patients having at least one sign and/or symptom of an ARI for ≤ 14 days in addition to a positive test (reverse transcription polymerase chain reaction [RT-PCR] or antigen) for SARS-CoV-2 within 10 days from symptom onset. ARI signs and/or symptoms included fever, cough, shortness of breath, loss of taste, loss of smell, use of respiratory support for the acute illness, and new pulmonary findings on chest imaging consistent with pneumonia. Test-negative controls were patients with at least one sign/symptom of ARI for ≤ 14 days and negative testing for SARS-CoV-2 by RT-PCR within 10 days of symptom onset. Syndrome-negative controls were patients who were admitted to hospital without ARI signs or symptoms and without any positive SARS-CoV-2 results from testing in the prior 14 days.

In addition to clinically obtained SARS-CoV-2 tests, enrolled participants had upper respiratory samples collected by enrolling staff and shipped to Vanderbilt University Medical Center for standardized, central laboratory RT-PCR testing for SARS-CoV-2. Patients enrolled as test-negative controls who tested positive for SARS-CoV-2 in the central laboratory were classified cases during analysis. Patients enrolled as syndrome-negative controls who tested positive for SARS-CoV-2 at the central laboratory were excluded from the analysis. An enrollment ratio of 1:1 for cases to controls was attempted at each site; controls were admitted within two weeks of cases per protocol.

Methods for classification of vaccination status and calculating VE in this program have been detailed in prior literature [24]. Briefly, vaccination information was obtained by participant self-report and systematic searches of clinical and public health sources, including CDC vaccination cards, state vaccine registries, and electronic health records. Analysis was restricted to patients vaccinated with two doses of an mRNA COVID-19 vaccine (BNT162b2 from Pfizer-BioNTech or mRNA-1273 from Moderna) ≥ 14 days prior to illness onset and unvaccinated patients receiving no doses of any COVID-19 vaccine. VE analyses were limited to 2 dose series of an mRNA vaccine due to small sample sizes of patients who received other vaccine types and the study period occurring before widespread use of third mRNA vaccine doses.

2.2. Statistical analysis

Participant characteristics were compared between the test-negative and syndrome-negative control groups using the chi square test for categorical variables and the Wilcoxon rank sum test for continuous variables. The characteristics included vaccination status, age (continuous and by subgroups 18–64 years and ≥ 65 years), sex, race/ethnicity (non-Hispanic White, non-Hispanic Black or African American, Hispanic, all other non-Hispanic), long-term care facility residence, health insurance, employment, education, number of hospitalizations in the prior year, number of categories of underlying medical conditions and immunosuppression status (Supplementary Table 1). Underlying medical conditions were grouped into seven categories (cardiovascular, neurologic, pulmonary, gastrointestinal, endocrine, renal, and hematologic). Participants were then classified by the number of categories their conditions were grouped under (0, 1, 2 or ≥ 3).

VE estimates for a two-dose series of mRNA vaccine to prevent COVID-19 hospitalization were calculated using each control group separately. VE was calculated using logistic regression models with case/control status as the outcome and vaccination status (fully vaccinated versus unvaccinated) as the primary exposure, while adjusting for potential confounding variables, including admission date (biweekly periods), age (continuous), sex, self-reported race and ethnicity, category of underlying conditions (0, 1, 2, or ≥ 3), immunosuppression status and US Health and Human Services region of enrolling hospital.

To evaluate VE by immunosuppressed versus immunocompetent status and by age group category (18–64 years versus ≥ 65 years), interaction terms were introduced into the adjusted VE models. VE was then calculated according to immunosuppression status within each age category using regression models that included interaction terms between vaccination status, age group, and immunosuppression status. Adjusted odds ratios (aORs) from these models were used to calculate VE using the formula: $VE = (1 - aOR) \times 100\%$. Statistical differences in VE across groups was evaluated using a standard two sample difference test, a more powerful alternative to the overlap test [25]. We assumed a correlation of 0.5 between VE results from the two control groups. Other levels of potential correlation (0.2 and 0.8) were considered, as well as no correlation. All analyses were performed using R (R Core Team, version 4.0.3, 2020). Study activities were reviewed by the CDC and conducted consistent with applicable federal law and CDC policy (45C.F.R. part 46.102(l)(2), 21C.F.R. part 56; 42 U.S.C. §241(d); 5 U.S.C. §552a; 44 U.S.C. §3501 et seq). These activities were determined to be public health surveillance with waiver of informed consent by institutional review boards at CDC and each enrolling site.

3. Results

3.1. Participants

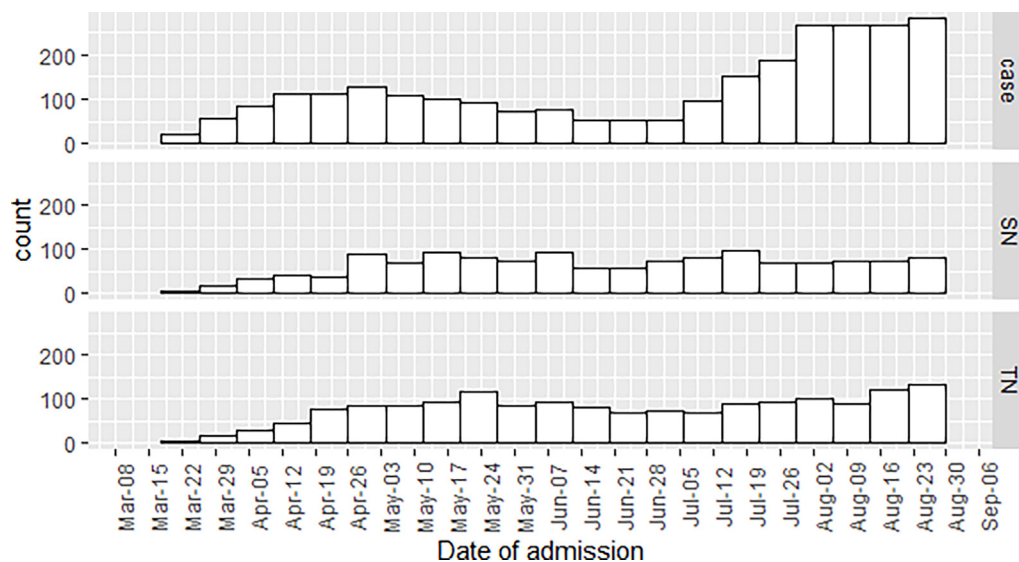
During the study period, 5,811 patients were identified as eligible for this analysis, including 2726 (46.9 %) COVID-19 case patients, 1389 (23.9 %) syndrome-negative controls, and 1696 (29.2 %) test-negative controls (Table 1). Median age overall was 59 years (IQR 45–69), 52 % identified as female, 23 % were non-Hispanic Black, 16 % Hispanic of any race, and 78 % had one or more underlying medical condition categories. Enrollment varied across study weeks, with case enrollment following similar trends as overall US COVID-19 activity (Fig. 1).

The percentage of participants vaccinated in the syndrome-negative control group (57 %) and test-negative control group (58 %) was similar ($P = 0.708$). However, control groups differed

Table 1

Participant characteristics of COVID-19 cases, syndrome-negative controls and test-negative controls enrolled by the IVY Network, March 11–August 31, 2021.

| Characteristic, no. (%) expect where otherwise stated | Case-patients | Syndrome-negative Controls | Test-negative Controls | P Value, syndrome-negative vs test-negative control |
|--|---------------|----------------------------|------------------------|---|
| Total enrolled | 2726 | 1389 | 1696 | |
| Vaccinated (2 mRNA COVID-19 vaccine doses) | 483 (17.7) | 790 (56.9) | 976 (57.5) | 0.708 |
| <i>Age group</i> | | | | |
| Median age in years (IQR) | 55 [42–67] | 60 [46–71] | 63 [50–72] | <0.001 |
| ≥65 years | 805 (29.5) | 566 (40.7) | 758 (44.7) | 0.028 |
| <i>Sex</i> | | | | |
| Female | 1312 (48.1) | 668 (48.1) | 840 (49.5) | 0.427 |
| <i>Race/Ethnicity</i> | | | | |
| White, non-Hispanic | 1321 (48.5) | 888 (63.9) | 1025 (60.4) | 0.033 |
| Black, non-Hispanic | 677 (24.8) | 277 (19.9) | 361 (21.3) | |
| Any race, Hispanic | 545 (20.0) | 167 (12.0) | 212 (12.5) | |
| All other races, non-Hispanic | 131 (4.8) | 39 (2.8) | 81 (4.8) | |
| Unknown | 52 (1.9) | 18 (1.3) | 17 (1.0) | |
| Residence in long-term care facility | 64 (2.4) | 50 (3.8) | 114 (6.9) | <0.001 |
| Has health insurance | 2463 (90.4) | 1298 (93.4) | 1606 (94.7) | 0.143 |
| Employed | 952 (44.3) | 382 (32.5) | 342 (23.6) | <0.001 |
| Employed as a health care worker | 134 (6.2) | 59 (5.0) | 62 (4.3) | 0.365 |
| Attended some college or more | 921 (50.9) | 576 (55.3) | 675 (52.1) | 0.129 |
| ≥1 hospital admission in past year | 714 (28.5) | 652 (49.5) | 925 (57.6) | <0.001 |
| <i>Number of categories of underlying medical conditions</i> | | | | |
| 0 categories of underlying conditions | 819 (30.0) | 285 (20.5) | 189 (11.1) | <0.001 |
| 1 categories of underlying conditions | 748 (27.4) | 421 (30.3) | 383 (22.6) | |
| 2 categories of underlying conditions | 635 (23.3) | 348 (25.1) | 510 (30.1) | |
| ≥3 categories of underlying conditions | 524 (19.2) | 335 (24.1) | 614 (36.2) | |
| <i>Specific underlying medical conditions</i> | | | | |
| Cardiovascular Disease | 1454 (53.3) | 850 (61.2) | 1224 (72.2) | <0.001 |
| Hypertension | 1338 (49.1) | 754 (54.3) | 1032 (60.8) | <0.001 |
| Endocrine Disease (Including Diabetes) | 880 (32.3) | 485 (34.9) | 698 (41.2) | <0.001 |
| Diabetes | 754 (27.7) | 389 (28.0) | 599 (35.3) | <0.001 |
| Pulmonary Disease | 588 (21.6) | 279 (20.1) | 636 (37.5) | <0.001 |
| COPD | 214 (7.9) | 145 (10.4) | 336 (19.8) | <0.001 |
| Renal Disease | 318 (11.7) | 216 (15.6) | 363 (21.4) | <0.001 |
| Hematologic Disease | 255 (9.4) | 174 (12.5) | 272 (16.0) | 0.006 |
| Neurological Disease | 215 (7.9) | 123 (8.9) | 195 (11.5) | 0.016 |
| Gastrointestinal Disease | 114 (4.2) | 120 (8.6) | 181 (10.7) | 0.058 |
| BMI-based obesity (≥30 kg/m²) | 1504 (55.9) | 526 (38.3) | 741 (43.9) | 0.002 |
| Immunosuppressed | 426 (15.6) | 260 (18.7) | 441 (26.0) | <0.001 |

**Fig. 1.** Counts of participants enrolled by the IVY Network, March 11–August 31, 2021 by week and enrollment group (cases, syndrome-negative (SN) controls, test-negative (TN) controls).

across a range of other baseline characteristics including age, race/ethnicity, residence in long term care facility, employment, one or more previous hospitalization in the past year, number of cate-

gories of and specific medical conditions, and immunosuppression. Test-negative controls, compared to syndrome-negative controls, were older (median age 63 versus 60 years, $P < 0.001$), and more

likely to be a resident of a long-term care facility (7 % versus 4 %, $P < 0.001$), have had at least one hospitalization within the past year (58 % versus 50 %, $P < 0.001$), have one or more categories of underlying medical conditions (89 % versus 79 %, $P < 0.001$), and more likely to be immunosuppressed (26 % versus 19 %, $P < 0.001$) (Table 1).

3.2. Vaccine effectiveness

VE estimates for a two-dose mRNA vaccine series to prevent COVID-19 hospitalization were similar when the test-negative and syndrome-negative control groups were used separately in this analysis (Fig. 2). Among immunocompetent patients aged 18–64 years, VE was 93 % (95 % CI: 90–94) with syndrome-negative controls and 91 % (95 % CI: 88–93) with test-negative controls. For immunocompetent patients aged ≥ 65 years, VE with syndrome-negative controls was 88 % (95 % CI: 84–91), and with test-negative controls was 84 % (95 % CI: 80–88). For immunosuppressed patients aged 18–64 years, VE was 59 % (95 % CI: 36–73) with syndrome-negative controls and 64 % (95 % CI: 48–75) with test-negative controls. VE among immunosuppressed patients aged 65 years and older was 53 % (95 % CI: 14–74) with syndrome-negative controls and 53 % (95 % CI: 22–72) with test-negative controls. Formal statistical tests assuming a true correlation assumption of 0.5 failed to reject the estimates being equal (all $P > 0.05$) with the exception of the eldest immunosuppressed group ($P = 0.04$). Tests using other correlation values showed similar results (Supplementary Table 2). Examination of point estimates among immunosuppressed patients aged 65 years and older does not suggest clinically important differences in VE estimates.

4. Discussion

Our results demonstrate that using hospitalized patients with ARI who test negative for SARS-CoV-2 (test-negative controls) and hospitalized patients without ARI (syndrome-negative controls) as control groups to estimate COVID-19 vaccine effectiveness produced very similar estimates of VE. Despite demographic and clinical differences between control groups, control-group-specific VE estimates were similar within age and immunosuppression status subgroups. Given comparable results with either con-

trol group, the IVY network did pool both controls to improve precision for subgroup analyses (product specific VE, age groups, immunosuppressed, chronic medical conditions) in prior publications. However, since October 2021, the IVY Network dropped additional enrollment of syndrome-negative controls based on logistical considerations and currently conducts VE analyses using only test-negative controls [23,24,26,27].

Among age and immunosuppression status subgroups, most statistical tests aligned with expert clinical thinking that VE estimates from the syndrome-negative control group and test-negative control group fail to reject the null hypothesis of equal estimates. However, the immunosuppressed patients aged 65 years and older yield VE estimates which indicate differences in results between the two control groups. The small sample size of these subgroups may contribute to these results.

Our findings on VE are consistent with other observational studies that evaluated outcomes of severe disease, including hospitalization and death [28,29]. However, while over 100 COVID-19 vaccine effectiveness studies to date have utilized TND, few have utilized two control groups [13]. To our knowledge, only one study has directly compared demographic characteristics and VE estimates using different control groups; however, all control groups met an eligibility requirement of symptomatic infection [30].

Four factors warrant consideration when interpreting these findings. First, when using test-negative controls, imperfect accuracy of diagnostic tests may lead to misclassification of case-control status which can, in turn, underestimate VE [17,21]. However, as SARS-CoV-2 RT-PCR tests used in the US are now highly sensitive and specific for samples collected early after ARI symptom onset, misclassification of SARS-CoV-2 status among syndrome-positive, test-negative control-patients is reduced [31,32]. Additionally, IVY defines eligible patients as those with testing within 10 days of symptom onset, reducing the likelihood of misclassification due to persistent positivity. Additionally, potential misclassification of the exposure due to ambiguity of onset of vaccine effect was further minimized in IVY by restricting vaccinated patients to those receiving both mRNA doses ≥ 14 days prior to illness onset. Syndrome-negative control groups are likely to be more important for illnesses without highly accurate diagnostic tests, as well as for evaluations of diseases with less severe outcomes.

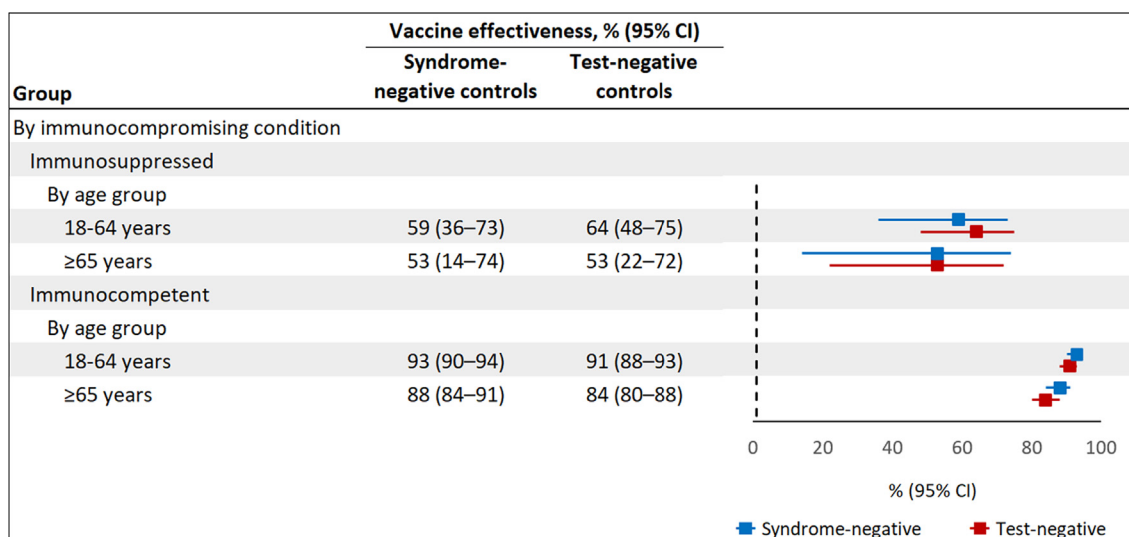


Fig. 2. Vaccine effectiveness against COVID-19 hospitalization for a two-dose series of COVID-19 mRNA vaccine, by age group and immunocompromised status, stratified by control group (syndrome-negative control versus test-negative control) of enrolled patients from IVY Network, March 11–August 31, 2021.

Second, VE studies are typically conducted among patients who seek healthcare; for example, in the IVY studies, VE of COVID-19 vaccines is estimated using patients who presented to a hospital and were admitted. Differences in healthcare seeking behavior, such as the propensity to present to a hospital, between cases and controls can bias estimates of VE [5,15,33]. Differential healthcare seeking behavior is suspected to be less severe for test-negative controls than syndrome-negative controls because, like cases, test-negative controls presented to the hospital for symptoms of an ARI. In contrast, syndrome-negative controls constitute a large and heterogeneous pool of patients with different medical conditions, each of which may have different patterns for healthcare-seeking behavior. Additionally, these patterns may vary over time as nonpharmaceutical COVID-19 interventions (e.g., social distancing, masking) are lessened, leading to a resurgence of other respiratory infections (e.g., influenza, respiratory syncytial virus) [18].

Third, the logistical challenges of enrolling controls are important for timely reporting of VE. Enrolling adequate sample size and collecting high quality data that accurately captures potential confounders, vaccine history, and laboratory outcomes is a time intensive endeavor. Test-negative controls ease logistical challenges of enrollment because they are enrolled in the same process of identifying case-patients through ARI surveillance. Thus, necessary information is likely routinely collected for test-negative controls, easing the overall cost, identification, and enrollment of such patients. Identifying syndrome-negative controls is further complicated by the large pool of potential patients, which might introduce certain selection biases and site-specific variability if not selected randomly (e.g., enrollment of patients from a specialty ward).

The findings in this report are subject to several limitations. The reported findings are most relevant for COVID-19 VE studies enrolling hospitalized adults. These findings may not generalize to other settings, such as to VE studies of other pathogens or studies in the outpatient environment. Second, control group selection for VE studies was only evaluated against COVID-19 hospitalization; therefore, studies assessing different outcomes (e.g., infection or death) may not be informed by our results. Third, test-negative controls were not tested for other viruses in this study. It is possible that patients positive for other viruses could offer additional advantages than virus-negative ARI controls.[34] Fourth, enrolling sites were academic medical centers, which tend to have patients with a high burden of chronic medical conditions and findings may not fully generalize to populations with lower burden of chronic disease. Fifth, this study was conducted during time periods of SARS-CoV-2 Alpha and Delta variant predominance. Differences between VE estimates obtained using test-negative and syndrome-negative control groups may be greater during periods of circulating variants associated with higher disease prevalence and/or lower clinical severity.

5. Conclusion

In this analysis, similar results were found for VE estimates for mRNA vaccines against COVID-19 hospitalization using test-negative and hospital-based syndrome-negative controls despite demographic and clinical differences between the two control groups. Imperfect diagnostic accuracy for SARS-CoV-2 tests does not appear to be introducing substantial bias into VE estimates against severe COVID-19 generated with the test-negative design and hospital-based controls with ARIs enrolled within two weeks of illness onset and tested within 10 days. These findings plus a large body of experience and evidence supporting test-negative controls for rotavirus and influenza VE studies suggest that use of test-negative controls in a test-negative design is a valid approach to estimating VE against severe COVID-19 resulting in hospitalization.

6. Disclaimer

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

7. Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

8. Notes

A full list of investigators and collaborators in the Influenza and Other Viruses in the Acutely Ill (IVY) Network is available in Appendix A in the Supplement.

9. Note

For the Influenza Vaccine Effectiveness in the Critically Ill (IVY) Investigators.

10. Ethics Approval

This evaluation was determined to be a public health surveillance program, with waiver of participant informed consent by all participating institutions and the Centers for Disease Control and Prevention (CDC). CDC and each participating institution reviewed and conducted this activity consistent with applicable federal law and CDC policy (45C.F.R. part 46.102(1)(2), 21C.F.R. part 56; 42 U.S.C. §241(d); 5 U.S.C. §552a; 44 U.S.C. §3501 et seq.).

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Data availability

No additional data available.

Declaration of Competing Interest

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.vaccine.2022.10.034>.

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