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Letters to the Editor

RE: "MEASUREMENT OF VACCINE DIRECT EFFECTS UNDER THE TEST-NEGATIVE DESIGN"

In a recent article, Lewnard et al. (1) presented a detailed examination of the test-negative design odds ratio. In the test-negative design, vaccine effectiveness is estimated as 1 minus the odds ratio. The authors considered 2 key vaccine protection models: "all-or-nothing," where a proportion of the vaccinated population is fully protected and the remaining population is fully susceptible, and "leaky," where the vaccine reduces the rate of infection in all vaccinated individuals. A central conclusion of the authors is that the test-negative design odds ratio is unable to recover an unbiased estimate of vaccine effectiveness under the leaky model.

An underappreciated fact about the test-negative design is that selection of test-negative controls naturally parallels density sampling (2). Controls emerge from the population at risk for the test-positive disease, and, given the central assumption that vaccine has no effect on other etiologies, these controls reflect the underlying distribution of exposure to vaccine. As a result, the odds ratio directly estimates the incidence rate ratio without requiring a rare disease assumption (3).

For density sampling to hold in a test-negative design, persons in the at-risk population must be able to repeatedly test negative (i.e., test-negative infections are not immunizing), and people must be censored from the at-risk population once they test positive. In the article by Lewnard et al. (1), the authors account for the first feature in their derivations but do not capture the second. Adopting the authors' notation, assuming that the vaccine is leaky with incidence rate ratio θ, the expected cumulative numbers of test-negative cases in vaccinated and unvaccinated participants, respectively, are

$$C_{VN}(t) = \lambda_N \pi_N \mu_V v P \frac{(1 - e^{-\theta\lambda_I t})}{\theta\lambda_I}$$

and

$$C_{UN}(t) = \lambda_N \pi_N \mu_U (1 - v) P \frac{(1 - e^{-\lambda_I t})}{\lambda_I}.$$

Here, t is replaced by the expected person-time at risk ($<t$), reflecting that persons in a density sampling design would be censored if they tested positive before time t (see Appendix). Importantly, these terms capture differential depletion of vaccinated and unvaccinated persons from the pool at risk for the test-negative disease. The test-negative design odds ratio (OR) is then

$$\begin{aligned}\mathrm{OR}(t) &= \frac{C_{VI}(t)C_{UN}(t)}{C_{UI}(t)C_{VN}(t)} \\ &= \frac{[\pi_I \mu_V (1 - e^{-\theta\lambda_I t}) v P]\left[\lambda_N \pi_N \mu_U (1 - v) P \frac{(1 - e^{-\lambda_I t})}{\lambda_I}\right]}{[\pi_I \mu_U (1 - e^{-\lambda_I t})(1 - v) P]\left[\lambda_N \pi_N \mu_V v P \frac{(1 - e^{-\theta\lambda_I t})}{\theta\lambda_I}\right]} \\ &= \theta.\end{aligned}$$

Thus, the test-negative design odds ratio is unbiased for a leaky vaccine when the study meets the criteria for density sampling. Practically, this means that participants 1) must be able to repeatedly test negative, 2) must be able to test negative and later test positive, and 3) must not be able to test negative after testing positive. This censoring structure, though simple, differs from previously recommended approaches (4). Besides yielding an unbiased estimator for vaccine effectiveness, this structure has other advantages, such as naturally accommodating changes in vaccine coverage over time, as would occur during an outbreak response.

Acknowledgments

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APPENDIX

Let $T_I \sim \text{Exponential}(\lambda_I)$ denote the random test-positive time for an unvaccinated individual. At study time t, individuals who have not tested positive will have person-time at risk t. Otherwise, individuals will have person-time at risk T_I, where $T_I \leq t$. The expected value of T_I conditional on $T_I \leq t$ is calculated as follows:

$$
\begin{aligned}
E[T_I|T_I \le t] &= \frac{1}{1-e^{-\lambda_I t}} \int_0^t u\lambda_I e^{-\lambda_I u} du \\
&= \frac{1}{1-e^{-\lambda_I t}} [-ue^{-\lambda_I u} - \frac{1}{\lambda_I} e^{-\lambda_I u}\Big|_{u=0}^{u=t} \\
&= \frac{1}{1-e^{-\lambda_I t}} \left[-te^{-\lambda_I t} - \tfrac{1}{\lambda_I} e^{-\lambda_I t} + \tfrac{1}{\lambda_I}\right] \\
&= \frac{1}{\lambda_I} - \frac{te^{-\lambda_I t}}{1-e^{-\lambda_I t}}.
\end{aligned}
$$

Thus, the expected person-time at risk for each unvaccinated individual by study time t is

$$
\begin{aligned}
&E[T_I|T_I \le t]\Pr(T_I \le t) + t\Pr(T_I > t) \\
&= \left[\frac{1}{\lambda_I} - \frac{te^{-\lambda_I t}}{1-e^{-\lambda_I t}}\right](1-e^{-\lambda_I t}) + te^{-\lambda_I t} \\
&= \frac{(1-e^{-\lambda_I t})}{\lambda_I} - te^{-\lambda_I t} + te^{-\lambda_I t} \\
&= \frac{(1-e^{-\lambda_I t})}{\lambda_I}.
\end{aligned}
$$

A parallel derivation is used for vaccinated individuals.

THE AUTHORS REPLY

We thank Dr. Dean for her comments (1). The misleading appearance of time-dependent vaccine effectiveness (VE) is a problem under diverse study designs that do not ascertain time to infection or do not ascertain all infections (2). In our article (3), we highlighted that VE estimates under the test-negative design (TND) may be exposed to time-dependent bias when the vaccine effect on infection is nonnull. Differential depletion of person-time at risk among vaccinated and unvaccinated persons who acquire natural immunity through undetected infections may create the appearance of waning immunity.

Dr. Dean proposes a modification to the TND aiming to correct for this bias in VE estimates by analyzing a subset of data sampled from vaccinated and unvaccinated persons' influenza-susceptible time at risk (1). Censoring observations from persons with a known history of influenzavirus infection is a compelling design-level correction for the problems created by differential preinfection person-time among the vaccinated and unvaccinated. We believe strategies such as this should be sought urgently to salvage valid inferences from TND studies that present numerous other advantages, such as efficiency and the ability to correct for health-care–seeking bias.

Nonetheless, we caution that there are feasibility considerations to take into account for the specific approach suggested by Dean (1). The proposed strategy requires that all influenzavirus infections (or at least those preceding a test-negative visit) be observed by researchers—in other words, that each influenzavirus infection result in a person's seeking care from the study team, receiving an influenza diagnostic test, and having his or her infection status recorded. However, approximately 65%–85% of influenza infections are clinically inapparent (4); even if symptoms occur, it remains the case that only a minority of people seek medical attention and an even smaller fraction receive diagnostic tests (5). While the proportion varies by age and setting, previous studies have suggested that surveillance of and testing for acute respiratory illnesses will capture only 1 out of every 79 (95% confidence interval: 47, 148) cases of symptomatic influenza not requiring hospitalization (6). The probability of detecting a previous influenzavirus infection, given that it occurred, is further reduced in TND studies that enroll only inpatients.

Various study designs have been undertaken to create data-collection streams that are amenable to such analyses for diseases with a high proportion of asymptomatic or mild infections. In the most notable instance, birth cohort studies coupling regular (weekly or biweekly) testing of asymptomatic stool for rotavirus infection with active surveillance of clinical gastroenteritis have yielded insights into the protective effect of previous rotavirus infection against future infection and disease (e.g., see Velázquez et al. (7)), but such designs necessitate prospective follow-up of participants, which the TND aims to circumvent.

What information, then, could enable researchers to censor naturally immune person-time in the retrospective context of TND studies, as Dean proposes (1)? We propose that testing for immunological evidence of recent influenza infection may present an attractive option. For instance, an assay which indicates recent infection (e.g., within the same season) and which discriminates between the response to natural infection and vaccination could provide a basis for censoring test-negative observations believed to have occurred after a naturally immunizing influenza infection. If it can be assumed that recent influenza infection deterministically prevents future infections, the non-censored observations will presumably represent samples from people's influenza-susceptible time at risk. Collection of sera for ascertainment of previous infection would impose less of a burden than prospective follow-up, although serological criteria for ascertaining recent infection should be considered carefully in the absence of a baseline specimen. The feasibility of this and other strategies, and any impact on inferences regarding influenza VE, should be explored in future studies.

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