

Invited Commentary

Invited Commentary: Assessing Mechanistic Interaction Between Coinfecting Pathogens for Diarrheal Disease

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The interaction estimates from Bhavnani et al. (*Am J Epidemiol.* 2012;176(5):387–395) are used to evaluate evidence for mechanistic interaction between coinfecting pathogens for diarrheal disease. Mechanistic interaction is said to be present if there are individuals for whom the outcome would occur if both of 2 exposures are present but would not occur if 1 or the other of the exposures is absent. In the epidemiologic literature, mechanistic interaction is often conceived of as synergism within Rothman's sufficient-cause framework. Tests for additive interaction are sometimes used to assess such synergism or mechanistic interaction, but testing for positive additive interaction only allows for the conclusion of mechanistic interaction under fairly strong "monotonicity" assumptions. Alternative tests for mechanistic interaction, which do not require monotonicity assumptions, have been developed more recently but require more substantial additive interaction to draw the conclusion of the presence of mechanistic interaction. The additive interaction reported by Bhavnani et al. is of sufficient magnitude to provide strong evidence of mechanistic interaction between rotavirus and *Giardia* and between rotavirus and *Escherichia coli*/Shigellae, even without any assumptions about monotonicity.

coinfecting pathogens; diarrhea; interaction; mechanism; synergism

Abbreviations: CI, confidence interval; ICR, interaction contrast ratio; RERI, relative excess risk due to interaction; RR, relative risk.

In their paper, Bhavnani et al. (1) have presented strong evidence of interaction, of a fairly substantial magnitude, between different coinfecting pathogens for diarrheal disease. Specifically, they report positive additive and multiplicative interaction between rotavirus and *Giardia* and between rotavirus and *Escherichia coli*/Shigellae. They also report positive additive and multiplicative interaction between *Giardia* and *E. coli*/Shigellae, although in this case, neither additive nor multiplicative interaction is statistically significant. In this invited commentary, the evidence for mechanistic interaction between these coinfecting pathogens is evaluated by using the results of their analyses.

ASSESSING MECHANISTIC INTERACTION

Suppose that we have 2 binary exposures and a binary outcome and that we let p_{ij} denote the probability of the outcome when the first exposure is i and the second exposure

is j . For now, for simplicity, we assume that there is no confounding for the effects of the exposures on the outcome, but we will return to this assumption later. Additive interaction using risks would typically be assessed by $(p_{11} - p_{00}) - \{(p_{10} - p_{00}) + (p_{01} - p_{00})\}$, which can be rewritten as

$$p_{11} - p_{10} - p_{01} + p_{00}. \quad (1)$$

This quantity assesses whether the effect of the 2 exposures together exceeds the sum of their effects considered separately. If the interaction contrast above is greater than 0, the additive interaction is said to be positive; if less than 0, then negative. Sometimes, instead, a ratio measure is used to evaluate additive interaction. If we divide the interaction contrast above by p_{00} , we obtain a quantity sometimes referred to as the "relative excess risk due to interaction" (RERI) (2, 3) or the interaction contrast ratio (ICR):

$$\text{RERI} = \text{RR}_{11} - \text{RR}_{10} - \text{RR}_{01} + 1,$$

where RR_{ij} is the risk ratio (RR) when the first and second exposures are i and j , respectively, compared with the reference group when both exposures are absent. This quantity, RERI or ICR, is one of the interaction measures used by Bhavnani et al. If RERI is greater than 0, then the interaction contrast for risks in equation 1 must also be positive; likewise, if RERI is less than 0, then the interaction contrast for risks in equation 1 must be negative. Thus, we can evaluate additive interaction using this ratio measure. When the outcome is rare so that the odds ratio approximates the risk ratio, we can approximately estimate RERI in a case-control study using odds ratios for the different exposure combinations. Alternatively, if we know the prevalence of the outcome, we could obtain risk ratios directly. Confidence intervals for RERI can be obtained with regression by using the delta method (4) or by using bootstrapping (5).

Often, instead, measures of multiplicative interaction are used. The measure of multiplicative interaction for risk ratios is $RR_{11}/(RR_{10} \times RR_{01})$. This measures whether the risk ratio for both exposures together exceeds the product of the risk ratios for each exposure considered individually. If this measure is greater than 1, then the multiplicative interaction is said to be positive; if less than 1, then negative. Both additive and multiplicative measures of interaction are sometimes referred to as “statistical interaction.”

We might instead consider more mechanistic forms of interaction. For example, we might consider whether there are some individuals for whom the outcome would occur if both exposures were present but for whom it would not occur if just one or the other exposure were present. This would be a form of “mechanistic interaction” in that, if both exposures are present, the outcome is turned “on”; if one or the other is absent, the outcome is turned “off.” If we let D_{ij} denote the outcome that would have occurred for an individual if the first exposure were i and the second exposure were j , then we would say that such mechanistic interaction is present if there are individuals such that $D_{11} = 1$ but $D_{10} = D_{01} = 0$. A response pattern of this type is sometimes referred to as a “sufficient cause interaction” (6, 7). Response patterns of this type imply synergism in Rothman’s sufficient-cause framework (7, 8).

Additive interaction is sometimes used to test for such mechanistic or sufficient cause interaction. However, having positive additive interaction only implies such sufficient cause interaction under additional assumptions. If it can be assumed that both exposures are never preventive for any individual (formally, if D_{ij} is nondecreasing in i and j for all individuals), then RERI greater than 0 implies the presence of a sufficient cause interaction (3, 6). In fact, in this case the interaction contrast in equation 1 provides a lower bound on the proportion of individuals manifesting such a sufficient cause interaction (9). The assumption that neither exposure can ever be preventive for any individual is sometimes referred to as a “monotonicity” assumption; in many contexts it is a strong assumption. In fact, we can test for sufficient cause interaction without this assumption (6, 8) by testing whether RERI is greater than 1. Although this is a stronger condition on the RERI, it allows for testing for sufficient cause interaction without assumptions about monotonicity.

More recently, an even stronger notion of mechanistic interaction has also been considered. We might, for example, consider whether there are any individuals for whom the outcome would occur if and only if both of the exposures are present ($D_{11} = 1$ but $D_{10} = D_{01} = D_{00} = 0$). This has been referred to as an “epistatic” or “singular” interaction (10, 11). We can also test for such epistatic or singular interaction using RERI. The condition of RERI greater than 2 suffices for this conclusion without any assumptions about preventive action/monotonicity; RERI greater than 1 suffices if at least one of the exposures is never preventive for any individual; and RERI greater than 0 suffices if both are never preventive (10, 11). Thus, positive additive interaction (or positive RERI) allows us to conclude the presence of such mechanistic interaction if we are willing to make monotonicity assumptions about the exposures’ never preventing the outcome for any individual, but we can test for mechanistic interaction without such assumptions by using more stringent conditions for RERI (such as $RERI > 1$ or $RERI > 2$). Similar conditions are also available when exposures are ordinal rather than binary (11, 12) or when antagonism (13) rather than synergism is in view.

ASSESSING MECHANISTIC INTERACTION BETWEEN COINFECTING PATHOGENS

Let us turn now to the analyses of Bhavnani et al. (1). Using age-standardized measures, Bhavnani et al. report that the risk ratio for diarrheal disease for rotavirus (in the absence of *Giardia*) is 2.63, that the risk ratio for *Giardia* (in the absence of rotavirus) is 1.13, but that the risk ratio when both rotavirus and *Giardia* are present is 10.72. This makes $RERI$ (or ICR) = $10.72 - 2.63 - 1.13 + 1 = 7.96$ (95% confidence interval (CI): 3.13, 18.92). We thus have substantial additive interaction. Moreover, the value of RERI itself (and in fact its entire 95% confidence interval) exceeds the value 2, suggesting strong evidence for mechanistic interaction (of both “sufficient cause” and “epistatic” varieties) even in the absence of any monotonicity assumptions.

Likewise, when Bhavnani et al. examine additive interaction between rotavirus and *E. coli*/Shigellae, the risk ratio for rotavirus (in the absence of *E. coli*/Shigellae) is 2.63, the risk ratio for *E. coli*/Shigellae (in the absence of rotavirus) is 1.64, and the risk ratio when both rotavirus and *E. coli*/Shigellae are present is 13.20. This gives a $RERI$ = $13.20 - 2.63 - 1.64 + 1 = 9.93$ (95% CI: 2.61, 28.41), and once again, the value of RERI itself and its entire 95% confidence interval exceeds the value 2, thus again suggesting strong evidence for mechanistic interaction (of both “sufficient cause” and “epistatic” varieties), in the absence of any monotonicity assumptions.

Finally, when Bhavnani et al. examine additive interaction between *Giardia* and *E. coli*/Shigellae, the risk ratio for *Giardia* (in the absence of *E. coli*/Shigellae) is 1.13, the risk ratio for *E. coli*/Shigellae (in the absence of *Giardia*) is 1.64, and the risk ratio when both *Giardia* and *E. coli*/Shigellae are present is 3.02. This gives a $RERI$ = $3.02 - 1.13 - 1.64 + 1 = 1.25$ (95% CI: -1.48, 3.13). In this case, the RERI estimate itself is greater than 1, which would

suggest sufficient cause interaction without monotonicity assumptions or “epistatic” interaction provided that at least 1 of the pathogens had a positive monotonic effect on diarrheal disease (i.e., was never preventive). However, here, the 95% confidence interval includes 0 and, thus, the overall evidence for mechanistic interaction between *Giardia* and *E. coli/Shigellae* is much weaker.

In the case of these pathogens (rotavirus, *Giardia*, and *E. coli/Shigellae*), the monotonicity assumption that the pathogens are never preventive of diarrheal disease may be quite plausible. However, in this case, the evidence for mechanistic interaction does require this assumption for interaction between rotavirus and *Giardia* and between rotavirus and *E. coli/Shigellae*, since the estimate and entire 95% confidence interval for RERI exceed 1 (the condition for sufficient cause interaction without monotonicity) and, in fact, even exceed 2 (the condition for epistatic interaction without monotonicity).

Some other measures related to RERI and additive interaction may also be of interest and are sometimes calculated. The attributable proportion defined by $RERI/RR_{11}$ captures the proportion of the risk of those with both exposures present that is attributable to the interaction itself. For example, with rotavirus and *Giardia*, the attributable proportion would be $RERI/RR_{11} = 7.96/10.72 = 74.2\%$. Alternatively, when both exposures are present, we could assess how much of the “excess relative risk” is due to the interaction by $RERI/(RR_{11} - 1) = 7.96/(10.72 - 1) = 7.96/9.72 = 81.9\%$; thus, of the move of the risk ratio from 1 (the reference category, both exposures absent) to 10.72 (when both exposures are present), 81.9% of this excess risk ratio of $10.72 - 1 = 9.72$ is due to the interaction. These are substantial proportions and are of considerable public health significance. Bhavnani et al. discuss the possibility of strategies for targeted rotavirus prevention. Among those with both rotavirus and *Giardia*, prevention of rotavirus would reduce the risk ratio from 10.72 to 1.13. The extent of the interaction gives rise to a dramatic risk reduction if one of the exposures can be prevented.

CONCLUDING REMARKS

Bhavnani et al. are to be commended for assessing additive interaction in their analyses. Although it has long been acknowledged that additive interaction is most relevant for assessing the public health significance of interaction (3, 14–16), estimates of additive interaction are still rarely reported in the epidemiologic literature (17); multiplicative interaction is presented with much more frequency. Bhavnani et al. also report multiplicative interaction and find that, for rotavirus and *Giardia*, this is 3.61 (95% CI: 1.33, 8.71); for rotavirus and *E. coli/Shigellae*, 3.06 (95% CI: 0.75, 7.27); and for *Giardia* and *E. coli/Shigellae*, 1.63 (95% CI: 0.47, 3.06). Only for rotavirus and *Giardia* does the 95% confidence interval not include 1. Power calculations to compare both additive and multiplicative interaction are now available (18), and whenever the main effects of both exposures are positive and the interaction is positive (the “classic” interaction pattern), the power for tests for additive interaction will exceed the power of those for multiplicative interaction

(18, 19). Assessing additive interaction is thus relevant both for assessing the public health significance of interaction and also because of potentially more powerful tests to assess interaction. Moreover, as we have seen above, additive interaction and RERI in particular can be useful in assessing mechanistic interaction. Arguably, whenever interaction is assessed, interaction on both the additive and multiplicative scales should be examined. The additive scale is perhaps often neglected because it is more difficult to obtain confidence intervals for such measures, but this can be done by using the delta method (4) or bootstrapping (5); SAS code (20) or standard logistic regression, along with an Excel spreadsheet (21), can fairly easily be used to carry this out. A variety of other methods for estimating RERI and confidence intervals are also available (22–26).

A couple of other comments and caveats merit attention. First, the tests and conditions described above for assessing mechanistic interaction assume that the effects of the exposures on the outcome are unconfounded conditional on the covariates. In their estimates of RERI, Bhavnani et al. (1) standardize their measures by age. Thus, if age sufficed to control for confounding of the effects of the pathogens on diarrheal disease, then the tests considered above for different forms of mechanistic interaction would be valid. However, we might be concerned that gender, diet, or number of children in the household, socioeconomic status, or some unknown variable might confound the exposure-outcome relation. Sensitivity analysis techniques are now available for assessing the extent to which unmeasured confounding can affect interaction analyses (27). In the case of the additive interaction between rotavirus and *Giardia* and between rotavirus and *E. coli/Shigellae*, the magnitude of the interaction is so great that very substantial confounding would be needed to change qualitative conclusions. However, in general, confounding needs to be thought about carefully in interaction analyses (28, 29), just as it does in other types of observational studies.

Finally, it should be noted that we have focused here on assessing so-called mechanistic interaction (e.g., the outcome occurs if both exposures are present but not if just one or the other is present). Such mechanistic interaction is distinct from statistical interaction; as we have noted, additive interaction can sometimes be used to test for such mechanistic interaction, but the conditions required are generally stronger than merely positive additive interaction. This mechanistic or sufficient cause interaction was sometimes in the past referred to as “biologic interaction” (2, 3, 30). There has, however, been a move away from using the term “biologic interaction” (31, 32), because neither statistical (33–36) nor even mechanistic/sufficient cause interaction (8, 11, 35) necessarily implies actual physical or functional interaction of the exposures. The term “mechanistic interaction” may thus be more appropriate since, as discussed above, the presence of both exposures effectively turns the outcome “on,” but the absence of one or the other turns the outcome “off.” Here, using the results of Bhavnani et al., we have seen that there is strong evidence for such mechanistic interaction between rotavirus and *Giardia* and between rotavirus and *E. coli/Shigellae* in their effects on diarrheal disease. Bhavnani et al. discuss a number of

possible biologic mechanisms that may involve particular forms of interaction. They note that the synergistic effects may be specific involving attachment and invasion of the intestinal epithelia by pathogens or nonspecific resulting from inflammation. Statistical analyses, even those considered here for mechanistic interaction, cannot in general distinguish between such possibilities; statistical inference is limited in what it can reveal about underlying biology. What it does reveal here, however, is that whatever that underlying biology might be, it is such that it gives rise to individuals for whom diarrhea occurs if both of 2 coinfecting pathogens are present but not if only 1 or the other is.

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