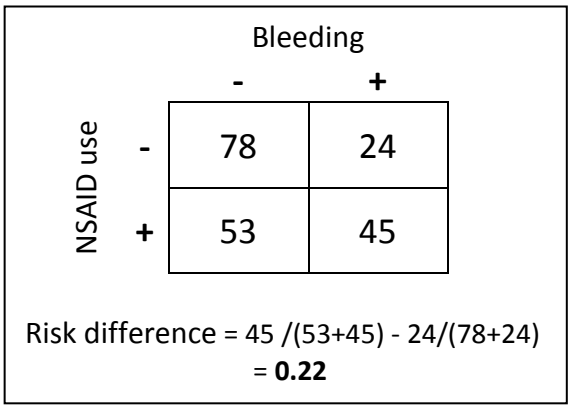
Appendix: Assessment of interaction and mediation

Assessment of effect modification

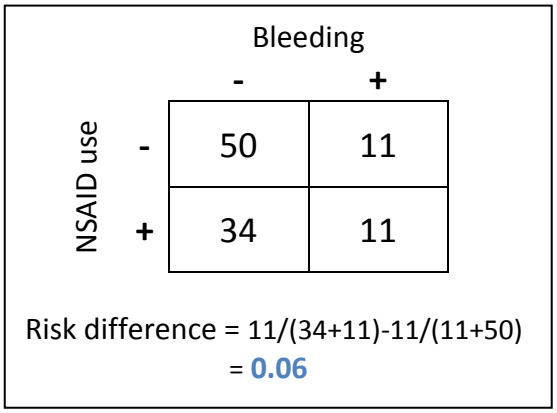
To obtain separate measures of effect based on a third variable used to define strata, the simplest and most widespread method is *stratification*. Using stratification, one computes the effect of *the exposure* on *the outcome* in each level, or *strata,* of the third variable. These stratum-specific effect measures *may* or *may not* equal one another.[1]

Here, we give a hypothetical example of an observational cohort study. This cohort study found that users of non-steroidal anti-inflammatory drugs (NSAIDs) had a risk difference of gastrointestinal bleeding of 22% compared with non-users of NSAIDs. To assess effect modification by sex, we stratify the overall analysis by sex categories (Appendix Figure 1). The risk difference of bleeding due to NSAID use after stratification was 6% in men *vs* 32% in women. As the risk differences did not equal one another, we say there was effect modification by sex. The test of heterogeneity of variances of a fraction of the total effect is a widely employed method in studies that assess effect modification. This method can be found in the work of Cochran.[2]

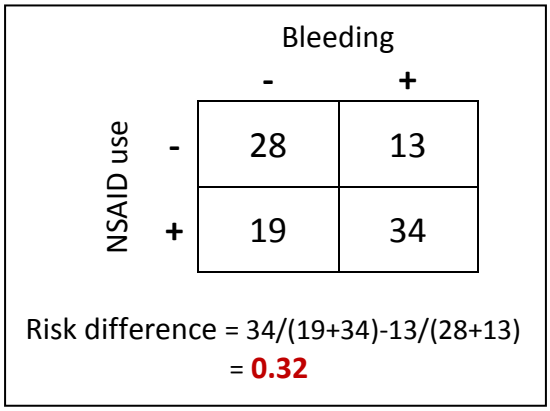


Stratification by *Sex*

Strata *sex* = 0, *men*



Strata *sex* = 1, *women*



Appendix Figure 1. Main effect and stratified analysis by sex from a hypothetical study.

Assessment of interaction

Interaction can be assessed using a variety of standard methods. The interaction contrast (IC)[1] assess departures from the sum of individual effects on a rate or a risk difference scale. Measures of relative excess risks due to interaction (RERI), synergy index (SI), and regression analyses are examples of methods that compute departure of the sum of individual effects on a relative risk, or ratio scale. We describe here the IC, the RERI and the regression analysis assessments. A description of the assessment of the synergy index can be found in the work of Rothman.[3]

1) Interaction Contrast

The interaction contrast (IC) is *the departure from uniform risk* or *rate differences* for a disease, due to interaction between two, or more, exposures.

Let us say we are interested in whether there is interaction between *A*, treatment with 'aspirin', and *B*, treatment with 'heparin', in determining an outcome, namely 'bleeding leading to hospitalization'. There is *interaction* between *A* and *B* if the *risk differences* for bleeding due to aspirin, ie, *A*, are *unequal* between individuals exposed and non-exposed to heparin, ie, *B*. This could be represented as:

*Risk difference due to **A** in **B**+* $\neq$ *Risk difference due to **A** in **B**-*

Appendix Table 1. One-year risk for 'bleeding leading to hospitalization', according to exposure status.

		Exposure B, *heparin* -	**Exposure B,** *heparin* +	
Exposure A, *aspirin*	-	0.1	0.4	- unexposed
	+	0.2	0.8	+ exposed

In this hypothetical example, using the values provided in Appendix Table 1, the risk difference for bleeding due to aspirin is 0.4 (0.8 *minus* 0.4) in individuals exposed to heparin, and 0.1 (0.2 *minus* 0.1) in individuals unexposed to heparin. As the *risk differences* for aspirin are *unequal* in those exposed and non-exposed to heparin, this indicates an *interaction* between aspirin and heparin.

The reverse situation is also valid, ie, there is *interaction* between *A* and *B* if the risk difference for heparin, ie, *B*, is unequal *between* subjects exposed *and* non-exposed to *A*:

$$(\text{Risk difference due to } \mathbf{B} \text{ in } \mathbf{A+}) \neq (\text{Risk difference due to } \mathbf{B} \text{ in } \mathbf{A-})$$

This *difference*, or *contrast*, between risk or rate differences is the IC:

$$IC = (\text{Risk difference due to } \mathbf{B} \text{ in } \mathbf{A+}) - (\text{Risk difference due to } \mathbf{B} \text{ in } \mathbf{A-})$$

Using the values from Appendix Table 1, *we obtain IC as:*

$$IC = (0.8 - 0.2) - (0.4 - 0.1) = 0.3$$

As the IC in this example is $\neq \mathbf{0}$, we say that there *is interaction* between *A* and *B*. Moreover, as the IC in this example is 0.3, and thus also exceeds **0**, we say that there *is synergistic interaction* between *A* and *B*. Conversely, an IC bellow 0 would imply an *antagonistic interaction* between *A* and *B*.

2) Relative excess risk due to interaction (RERI)

Interaction on a relative or ratio scale, or *RERI*, can be computed by dividing the IC to the risk, or rate, in the jointly non-exposed (patients exposed neither to *A* nor *B*), usually described as the *baseline risk*[1]:

$$RERI = \frac{IC}{Risk\ in\ the\ non-exposed}$$

Using the same example of the aspirin and heparin join exposures from the Appendix Table 1, we can obtain RERI as:

$$RERI = {}^{0.3}/_{0.1} = 3.0$$

As the RERI is this example **is not equal to 1**, we say that there is *interaction* between *A* and *B in a relative risk, or ratio scale*. As the RERI was above 1, the interaction was synergic. The RERI can be interpreted as there is a three-fold excess risk of bleeding due to the jointly exposure to aspirin and heparin. Conversely, an RERI below 1 would suggest an antagonistic interaction.

3) Regression modeling

Regression modeling can be used to assess interaction as well. As summarized by Greenland: *"In statistics, an interaction in a regression model is nothing more than a product term".*[4] By including a product term in a regression model, one normally identifies or not interaction using the likelihood ratio test.[5] Since almost all regression models in epidemiology are ratio scale models, the likelihood ratio test the null hypothesis of no departure from the sum of individual effects *in a relative risk or ratio scale*, as they imply in transforming estimates in a logarithm scale.

To obtain the *departure of effects on a relative risk or ratio scale*, instead of a product term, one would have to obtain the relative risk or risk ratio, i.e., using their regression coefficients, for those: exposed only to *A*, exposed only to *B*, and exposed to both *A* and *B*, **all** relative to the double non-exposed (Appendix Table 1), using the underlying assumption that the relative risk or risk ratio in the double non-exposed category (reference category) is equal to 1:

$$RERI = Relative\ risk_{\substack{A_+ \\ B_+}} - Relative\ risk_{\substack{A_- \\ B_+}} - Relative\ risk_{\substack{A_+ \\ B_-}} + 1$$

Appendix Table 2. Analysis of oral contraceptive use, presence of Factor V Leiden allele, and Odds ratio for venous thromboembolism

Factor V Leiden	Oral Contraceptives	N. patients	N. controls	Odds ratio
+	+	25	2	34.7
+	-	10	4	6.9
-	+	84	63	3.7
-	-	36	100	1 *(reference category)*

Results from *Botto & Khoury, 2001*[6]

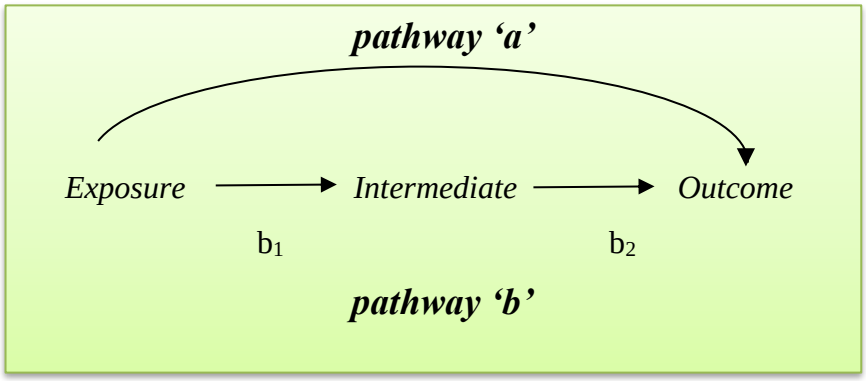
Using the Odds ratio estimates from Appendix Table 2, one can compute the RERI as (34.7) – (6.9) – (3.7) + 1 = **25.1**. Mind that the RERI obtained from estimates arising from regression models, do not hold the linearity assumption underneath the theoretical concept of the RERI estimation.[7]

Meaning of interaction assessments

As clinical researchers, most motivations previously described to assess interactions are inherently based on a wish to give a 'biologic' meaning for the results. No method to assess interaction can fulfill that wish.[4,8] It might be argued that the two exposures do not physically act on each other, and thus do not interact in any biological sense. Besides, one might be puzzled whether to assess interaction using departures from risk or rate differences, or from relative risk or ratios. The most 'meaningful' measures of interaction are tied to departures of effects on a risk or rate difference scale. From computing interaction on a risk or rate difference scale, it is possible to obtain estimates that allow us to "act".[9] For example, a hypothetical IC of 40 per 1,000 person-years was found in the assessment of interaction between treatment with aspirin and heparin on the increased risk of bleeding. This estimate could be interpreted as 40 new hospitalizations due to bleeding for every 1,000 patients per year could be potentially prevented, by avoiding the joint exposure to *heparin* and *aspirin*. This information could be easier to interpret if *preventing* or *intervening* on a disease is being contemplated.[10]

Assessment of mediation

Let us think about a hypothetical situation on which an *intermediate* can explain an association between an exposure and an outcome. In this situation, the simplest possible chain of events from exposure to disease, or outcome, is represented on Appendix Figure 3.



Appendix Figure 3. Possible pathways in the relation among the exposure, the intermediate, and the outcome

In this representation, the effect between *the exposure* and *the outcome* could be disentangled into:

- the effect that *acts* through *the intermediate* (***pathway 'b'***); and
- the effect that is *unexplained* by *the intermediate* (***pathway 'a'***)

There are two simpler, standard methods to identify mediation and obtain *direct* and *indirect effects*: the *difference method* and the *product method*.[11]

1) Assessment of mediation using the difference method

One of the first and simplest approaches to identify mediation is the difference, or change in estimate method. Basically, the difference method consists in performing two regression models*: one with and one without* the inclusion of the intermediate variable. If there is a *change* in the exposure-outcome estimate between these two models, then some of the effect is thought to be mediated.

Let's assume that an exposure *X*, an intermediate *I* and an outcome *Y* are continuous variables. To assess mediation using the difference method, we perform 2 linear models regressing *Y* on *X*, *with* and *without* the inclusion of the intermediate *I*:

$$E\,(Y|X) = \alpha_0 + \alpha_1 X \quad (1)$$

$$E\,(Y|X, I) = \theta_0 + \theta_1 X + \theta_2 I \quad (2)$$

From these two models, we obtain two coefficients for *X*: $\boldsymbol{\alpha_1}$ and $\boldsymbol{\theta_1}$. If these *differ*, then some of the effect is thought to be mediated. The difference of coefficients is used to obtain the *indirect effect.* Using equation (2), the coefficient $\boldsymbol{\theta_1}$ is used to obtain the *direct effect* (Appendix Table 3).

Appendix Table 3. Direct, indirect and total effects as assessed using the difference of coefficients method and the product of coefficients method

Type of effect	Pathway *(Figure 3)*	Measure of effect	
		Difference method	Product method
Direct effect	*a*	θ_1	θ_1
Indirect effect	*b*	$\alpha_1 - \theta_1$	$\beta_1 \times \theta_2$
Total effect	-	α_1	$\theta_1 + (\beta_1 \times \theta_2)$

2) Assessment of mediation using the product method

Let's again assume that *X*, *I* and *Y* are continuous variables. Similarly to the difference method, the product method is also assessed by obtaining estimates of effect from two regression models.[11] The first model is identical to the one described in equation (2), and it is also aimed to obtain the *direct effect* by the coefficient $\boldsymbol{\theta_1}$ (Appendix Table 3). However, in a second model, we regress *I* on *X*, as represented in the linear regression equation as:

$$E\,(I|X) = \beta_0 + \beta_1 X \qquad (3)$$

Using equation (3), with represents the pathway $\boldsymbol{b_1}$ from Appendix Figure 3, we obtain the coefficient $\boldsymbol{\beta_1}$. Using again equation (2), which represents the pathway $\boldsymbol{b_2}$, from Appendix Figure 3, we obtain the coefficient $\boldsymbol{\theta_2}$. By obtaining a product of the $\boldsymbol{\theta_2}$ and $\boldsymbol{\beta_1}$ coefficients, one can obtain the *indirect effect* (Appendix Table 3).

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