COMPARISON OF THREE MEDIATION ANALYSIS METHODS WITH
TWO SEQUENTIAL MEDIATORS

By

Xinchun Zhang

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ABSTRACT

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Mediation analysis is an important tool for understanding causal mechanisms in epidemiology and social sciences. The estimation of direct and indirect effects with multiple mediators is a challenging problem. This thesis focused on the comparison of three mediation analysis methods with two sequential mediators. Our goal was to access the robustness of the methods in estimating natural indirect effect and partial indirect effect. In this thesis we simulated multiple scenarios based on a counterfactual framework and employed three weighted-marginal structural models to estimate direct and indirect effects (1-3). The bias, root mean squared error and 95% confidence interval coverage probability from the Monte-Carlo simulations were the criteria to compare the three methods. By comparing their performance in the estimation of direct and indirect effects, we concluded that the Lange method was more robust in mediation analysis with two sequential mediators compared with the methods by Steen and Hong.

Key words: Causal inference, mediation analysis, sequential mediators, marginal structural models, data simulation, causal directed acyclic graph, direct effect, indirect effect

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CHAPTER 1. INTRODUCTION

Mediation analysis is used in epidemiology and social sciences to estimate how an exposure is related to an outcome through a mediator in complex observational settings. There may be a single mediator, or a set of mediators that are causally related between the exposure and the outcome. In many epidemiological studies, multiple mediators may be of interest. We are interested in assessing the extent to which the effect of an exposure on an outcome is mediated by two sequential mediators.

There have been two different statistical techniques—methods based on regression and methods based on weighting—to estimate direct and indirect effects in mediation analyses with multiple mediators. Regression-based approaches involve combination of results from two models, a model for the outcome and a model for the mediator, to estimate direct and indirect effects (4-6). The approaches described here work when all mediators are continuous, but cannot accommodate binary or categorical mediators, especially when these mediators interact. An alternative class of strategies for these cases is weighting-based methods. Weighting-based methods can be used more generally to setting with continuous, binary, count or time-to-event outcomes (7, 8). These approaches involve specifying exposure and mediator weights. Using a weighting approach, it is easy to overcome the difficulties in estimating direct and indirect effects with more than one mediator (5, 6, 8).

Following Liu et al. (9), we considered a mediation analysis with two sequential mediators. We employed weighting approaches, because the mediators are sequential.

Moreover, there were exposure-mediator and mediator–mediator interactions, which makes it difficult to obtain easily generalizable analytic expressions for the direct and indirect effects using regression-based approaches (2, 3, 10, 11).

Identification and estimation of unbiased direct and indirect effects rely on many assumptions, such as, no measurement error in the exposure, mediators, or outcome; no unaccounted-for confounding between exposure and mediator, exposure and outcome, or mediator and outcome; correct specification of the regression models for exposure-mediator-outcome relations (2, 3, 12). In the case of multiple mediators, mediation analysis methods using a potential outcomes framework have been proposed (12-14). Natural direct and indirect effects (NDE and NIE) were estimated using linear structural equation modeling, outcome and mediator regression-based methods, inverse-probability-of-treatment weighting (IPTW) fitting of marginal structural models (MSMs) (2, 3).

Several propensity score-based weighting methods for mediation analysis with multiple mediators have been developed. These methods apply the estimated weights rather than the true weights that are usually unknown, such as IPTW, and ratio-of-mediator-probability weighting (RMPW) (1, 7). Causal mediation analysis through IPTW or RMPW is a weighting-based approach to estimating NDE, and NIE through mediators. In this study, we compare Lange method (2), Steen method (3) and Hong method (1) in the causal mediation analysis with two sequential mediators.

The thesis is organized as follows: chapter 1-introduction; chapter 2-counterfactual approach to mediation analysis with two sequential mediators; chapter 3-the proposed simulation procedure; chapter 4-data simulation; chapter 5-simulation results; finally, chapter 6-conclusion.

CHAPTER 2. COUNTERFACTUAL APPROACH TO MEDIATION ANALYSIS WITH TWO SEQUENTIAL MEDIATORS

2.1 Motivating epidemiology example

For illustrative purposes, we revisit previous analyses on a cohort study about the connection between poor olfaction and mortality among older adults (9). As shown in Liu et al. (9), the effect of olfaction impairment on the risk of higher mortality among older adults were mediated by neurodegenerative diseases and weight loss. Previous mediation analysis suggested that neurodegenerative disease and weight loss may partly explain the relationship between poor olfaction and higher mortality (9, 15). In the thesis, all variables including the exposure, the outcome, two mediators, and six confounders, are binary variables. Corresponding natural direct and indirect effects are estimated under the assumption that baseline covariates are sufficient to control for confounding so that the identification assumptions are met (1, 2, 10, 16). The causal diagram of Figure 1 depicts a generalization of the causal relations between the aforementioned variables.

2.2 The counterfactual framework

The potential outcomes framework has been used to address mediation analysis (12, 17). This framework not only gives clear relationships among variables, but also defines mediation effects in causal terms. From the framework, researchers can not only reveal explicitly assumptions required for causal inference, but also formulate the confounding control needed for the direct and indirect causal effects of interest (3, 12, 16).

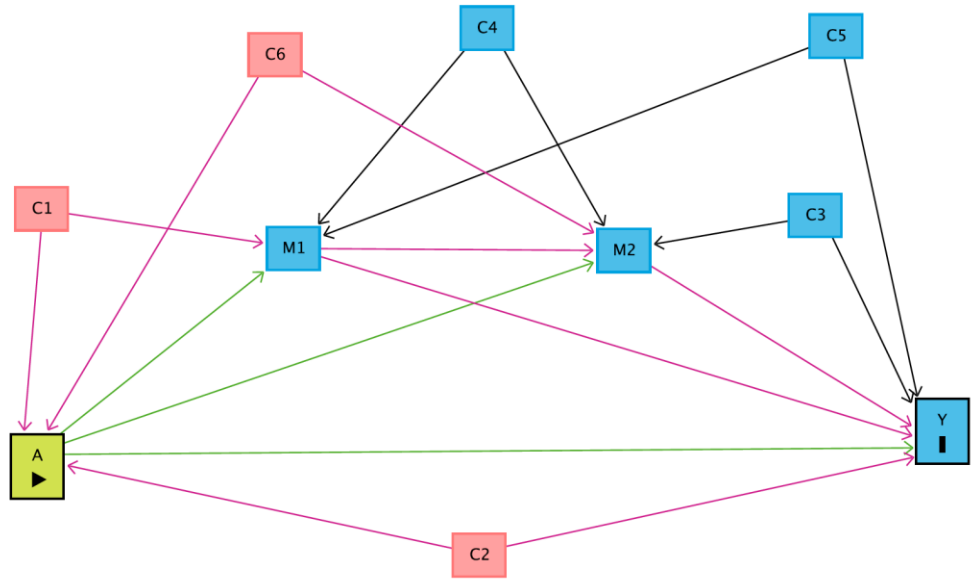

Figure 1. Causal directed acyclic graph (DAG) with exposure A, outcome Y, two sequential mediators M_1 and M_2, and a set of baseline confounders $C_1 - C_6$ sufficient for confounding control.

Note: The DAG was created using http://www.dagitty.net/dags.html.

We consider a cohort study in which Y denotes mortality among older adults (the outcome of interest), A denotes olfaction impairment (the exposure), M_1 and M_2 denote neurodegenerative diseases and weight loss (two sequential mediators) respectively. M_1 and M_2 are two causally ordered mediators, which means M_1 can affects M_2, but not vice versa. We allow for potential $\mathrm{A}\rightarrow\mathrm{M}_1/\mathrm{M}_2$ interactions as well as $\mathrm{M}_1\rightarrow\mathrm{M}_2$ interaction. C denotes a set of pre-exposure confounding covariates sufficient for confounding control. Under no-omitted-confounder assumption, $\{\mathrm{C}_1, \mathrm{C}_2, \mathrm{C}_6\}$ is the minimum sufficient adjustment set (MSAS) of $\mathrm{A}\rightarrow\mathrm{Y}$ relationship, C_1 is the MSC of $\mathrm{A}\rightarrow\mathrm{M}_1$ relationship, $\{\mathrm{A}, \mathrm{C}_3, \mathrm{C}_4, \mathrm{C}_6, \mathrm{M}_1\}$ and $\{\mathrm{A}, \mathrm{C}_2, \mathrm{C}_3, \mathrm{C}_5, \mathrm{M}_1\}$ are MSAS's of $\mathrm{M}_2 - \mathrm{Y}$ relationship, $\{\mathrm{A}, \mathrm{C}_1, \mathrm{C}_4\}$

and $\{A, C_4, C_6\}$ are MSAS's of $M_1 \rightarrow M_2$ relationship, $\{A, C_1, C_4, C_5\}$ is the MSAS of $M_1 \rightarrow Y$ relationship, $\{C_1, C_6\}$ is the MSAS of the $A \rightarrow M_2$ relationship (Table 1).

Table 1. Minimal sufficient adjustment sets (MSAS)

Exposure	Outcome	MSAS for estimating the total effect of exposure on outcome	MSAS for estimating the direct effect of exposure on outcome
A	Y	C_1, C_2, C_6	C_2, C_3, C_5, M_1
A	M_1	C_1	C_1
A	M_2	$C_1 C_6$	C_4, C_6, M_1
M_1	M_2	A, C_1, C_4	A, C_1, C_4 A, C_4, C_6
M_2	Y	$A, C_1, C_2, C_3, C_4, M_1$ $A, C_1, C_3, C_5, C_6, M_1$ A, C_2, C_3, C_5, M_1 A, C_3, C_4, C_6, M_1	$A, C_1, C_2, C_3, C_4, M_1$ $A, C_1, C_3, C_5, C_6, M_1$ A, C_2, C_3, C_5, M_1 A, C_3, C_4, C_6, M_1
M_1	Y	A, C_1, C_4, C_5 A, C_2, C_4, C_5, C_6	$A, C_1, C_3, C_5, C_6, M_2$ A, C_2, C_3, C_5, M_2

Note: This table was created using http://www.dagitty.net/dags.html.

2.3 Decomposition of causal effects

2.3a Counterfactual notation for natural direct and indirect effects

Let A = 1 if a subject is assigned to the exposed condition, and let A =0 if the same subject is assigned to the control condition instead (11, 12). Y^a is counterfactual or potential outcome when A is set to a. In this thesis, A is binary, then each subject has two potential outcomes: Y^0 and Y^1. When there is only one mediator M, the natural direct effect (NDE) measures how much Y would change if A were set at $a = 1$ versus $a^* = 0$, but for each subject M was kept at the $a^* = 0$. The natural indirect effect (NIE) estimates how much

Y would change if A were controlled at $a = 1$, but the counterfactual M were changed from $M^{a^* = 0}$ to $M^{a = 1}$, in which A were changed from level $a^* = 0$ to level $a = 1$.

We now consider the situation illustrated in Figure 1 which includes two causally sequential mediators M_1 and M_2. We use 3-way decompostion approach to identify the causal effects (3, 5). There are four finest possible distinct deposition from A to Y: $A \rightarrow Y$, $A \rightarrow M_1 \rightarrow Y$, $A \rightarrow M_2 \rightarrow Y$ and $A \rightarrow M_1 \rightarrow M_2 \rightarrow Y$.

M_1^1 denotes the potential value of the mediator M_1 under $a = 1$, M_1^0 represents the potential outcome of M_1 when $a^* = 0$. Then, $Y^{1, {M_1}^1, {M_2}^{1, {M_1}^1}}$ indicates the counterfactual value of Y that would be observed if A was assigned a=1, M_1 was set to value of M_1^1 that would be observed if A was set to a=1, M_2 was set to value of M_2^{1,M_1^1} that would be observed if A was set to a=1 and $M_1 = M_1^1$. For the same subject, when assigned to $a = 0$ instead, counterfactual outcome would be $Y^{0, {M_1}^0, {M_2}^{0, {M_1}^0}}$.

2.3b Decomposition in a mediation model with two sequential mediators

The causal mediation effects for a binary outcome can be presented in either the risk difference scale (RD) or the risk ratio scale (RR) (18). The total effect (TE) is defined as how much Y would change overall for a change in A from level $a^* = 0$ to level $a = 1$. In this counterfactual-based approach, the total effect decomposes into the natural direct and indirect effects (3).

If there are k sequential mediators in a DAG, the possible decompositions would be

$(k+1)$! Without imposing parametric restrictions. We employed Steen's natural effect models in a 3-way decomposition (3). The saturated model for a 3-way decomposition of causal effects with two sequential mediators M_1 and M_2 is:

$E(Y^{a, M_1{}^{a'}, M_2{}^{a'', M_1{}^{a'}}}) =$

$\beta_0 + \beta_1 * a + \beta_2 * a' + \beta_3 * a'' + \beta_4 * a * a' + \beta_5 * a * a'' + \beta_6 * a' * a'' + \beta_7 * a * a' * a''$,

where $a,\ a', a''$ are binary (coded 0 or 1).

Then, the direct effect of A on Y: $(a' = 0, a'' = 0)$

$$E_{A\to Y}(a', a'') = E_{A\to Y}(0,0) = g(E(Y^{1, M_1{}^0, M_2{}^{0,0}})) - g(E(Y^{0, M_1{}^0, M_2{}^{0,0}}))$$

$$= \beta_1 + \beta_4 * a' + \beta_5 * a'' + \beta_7 * a' * a'' = \beta_1$$

The indirect effect mediated by M_1 on Y: $(a = 1, a'' = 1)$

$$E_{A\to M_1 Y}(a, a'') = E_{A\to M_1 Y}(1,1) = g(E(Y^{1, M_1{}^1, M_2{}^{1, M_1{}^1}})) - g(E(Y^{1, M_1{}^0, M_2{}^{1, M_1{}^0}}))$$

$$= \beta_2 + \beta_4 * a + \beta_6 * a'' + \beta_7 * a * a'' = \beta_2 + \beta_4 + \beta_6 + \beta_7$$

The partial indirect effect mediated solely by M_2 (bypassing M_1) on Y: $(a = 1, a' = 0)$

$$E_{A\to M_2\to Y}(a, a') = E_{A\to M_2\to Y}(1,0) = g(E(Y^{1, M_1{}^0, M_2{}^{1, M_1{}^0}})) - g(E(Y^{1, M_1{}^0, M_2{}^{0, M_1{}^0}}))$$

$$= \beta_3 + \beta_5 * a + \beta_6 * a' + \beta_7 * a * a' = \beta_3 + \beta_5$$

In this study, we have six (3!) possible decompositions as shown below:

$$E_{A\to Y}(0,0) + E_{A\to M_1 Y}(1,1) + E_{A\to M_2\to Y}(1,0) = (\beta_1) + (\beta_2 + \beta_4 + \beta_6 + \beta_7) + (\beta_3 + \beta_5)$$

$$E_{A\to Y}(1,1) + E_{A\to M_1 Y}(0,0) + E_{A\to M_2\to Y}(0,1) = (\beta_1 + \beta_4 + \beta_5 + \beta_7) + (\beta_2) + (\beta_3 + \beta_6)$$

$$E_{A\to Y}(0,0) + E_{A\to M_1 Y}(1,0) + E_{A\to M_2\to Y}(1,1) = (\beta_1) + (\beta_2 + \beta_4) + (\beta_3 + \beta_5 + \beta_6 + \beta_7)$$

$$E_{A\to Y}(1,1) + E_{A\to M_1 Y}(0,1) + E_{A\to M_2\to Y}(0,1) = (\beta_1 + \beta_4 + \beta_5 + \beta_7) + (\beta_2 + \beta_6) + (\beta_3)$$

$$E_{A\to Y}(0,1) + E_{A\to M_1 Y}(1,1) + E_{A\to M_2\to Y}(0,0) = (\beta_1 + \beta_5) + (\beta_2 + \beta_4 + \beta_6 + \beta_7) + (\beta_3)$$

$$E_{A\to Y}(1,0) + E_{A\to M_1 Y}(0,0) + E_{A\to M_2\to Y}(1,1) = (\beta_1 + \beta_4) + (\beta_2) + (\beta_3 + \beta_5 + \beta_6 + \beta_7)$$

2.3c Computing true effects

The estimation of true effects is presented as follows based on Steen and Lange methods (2, 3).

The difference between the following two counterfactual outcomes is the total risk difference (RD) of treatment effect on the outcome ($\mathrm{TE_{RD}}$):

$$\mathrm{TE_{RD}} = \mathrm{E}(\mathrm{Y}^{1,\, \mathrm{M_1}^1,\, \mathrm{M_2}^{1,\ \mathrm{M_1}^1}}) - \mathrm{E}(\mathrm{Y}^{0,\, \mathrm{M_1}^0,\, \mathrm{M_2}^{0,\ \mathrm{M_1}^0}})$$

Natural direct effect (NDE) A on Y is a causal effect medicated not throught $\mathrm{M_1}$ or $\mathrm{M_2}$, but by pathway $A \to \mathrm{Y}$. NDE is defined as follows:

$$\mathrm{NDE_{RD(0,0)}} = \mathrm{E}(\mathrm{Y}^{1,\, \mathrm{M_1}^0,\, \mathrm{M_2}^{0,\ \mathrm{M_1}^0}}) - \mathrm{E}(\mathrm{Y}^{0,\, \mathrm{M_1}^0,\, \mathrm{M_2}^{0,\ \mathrm{M_1}^0}})$$

Natural indirect effect (NIE) captures all pathways mediated by $\mathrm{M_1}$:

$$\mathrm{NIE_{RD(1,1)}} = \mathrm{E}(\mathrm{Y}^{1,\, \mathrm{M_1}^1,\, \mathrm{M_2}^{1,\ \mathrm{M_1}^1}}) - \mathrm{E}(\mathrm{Y}^{1,\, \mathrm{M_1}^0,\, \mathrm{M_2}^{1,\ \mathrm{M_1}^0}})$$

A partial indirect effect (PIE) with respect to $\mathrm{M_2}$ as the mediator. PIE captures pathway: $\mathrm{A} \to \mathrm{M_2} \to \mathrm{Y}$. PIE is the indirect treatment effect on Y mediated by $\mathrm{M_2}$ bypassing $\mathrm{M_1}$:

$$\mathrm{PIE_{RD(1,0)}} = \mathrm{E}(\mathrm{Y}^{1,\, \mathrm{M_1}^0,\, \mathrm{M_2}^{1,\ \mathrm{M_1}^0}}) - \mathrm{E}(\mathrm{Y}^{1,\, \mathrm{M_1}^0,\, \mathrm{M_2}^{0,\ \mathrm{M_1}^0}})$$

For Hong method, decomposition of $\mathrm{TE_{RD}}$ and $\mathrm{NDE_{RD}}$ remain the same as aforementioned. However, $\mathrm{NIE_{RD}^{H}}$ and $\mathrm{PIE_{RD}^{H}}$ are different as discussed in Hong's book (1):

$$\mathrm{TE_{RD}} = \mathrm{E}(\mathrm{Y}^{1,\, \mathrm{M_1}^1,\, \mathrm{M_2}^{1,\ \mathrm{M_1}^1}}) - \mathrm{E}(\mathrm{Y}^{0,\, \mathrm{M_1}^0,\, \mathrm{M_2}^{0,\ \mathrm{M_1}^0}})$$

$$\mathrm{NDE_{RD(0,0)}} = \mathrm{E}(\mathrm{Y}^{1,\, \mathrm{M_1}^0,\, \mathrm{M_2}^{0,\ \mathrm{M_1}^0}}) - \mathrm{E}(\mathrm{Y}^{0,\, \mathrm{M_1}^0,\, \mathrm{M_2}^{0,\ \mathrm{M_1}^0}})$$

$$\mathrm{NIE_{RD(1,1)}^{H}} = \mathrm{E}(\mathrm{Y}^{1,\, \mathrm{M_1}^1,\, \mathrm{M_2}^{1,\ \mathrm{M_1}^1}}) - \mathrm{E}(\mathrm{Y}^{1,\, \mathrm{M_1}^0,\, \mathrm{M_2}^{1,\ \mathrm{M_1}^1}})$$

$$\mathrm{PIE}^{\mathrm{H}}_{\mathrm{RD}(1,0)} = \mathrm{E}(\mathrm{Y}^{1,\, \mathrm{M_1}^0,\, \mathrm{M_2}^{1,\ \mathrm{M_1}^1}}) - \mathrm{E}(\mathrm{Y}^{1,\, \mathrm{M_1}^0,\, \mathrm{M_2}^{0,\ \mathrm{M_1}^0}})$$

2.4 Assumptions that permit identification

Sufficient assumptions (1-3) for the identification of unbiased causal direct and indirect effects include:

(a) Consistency of A on Y: $\mathrm{Y}^{\mathrm{a}} = \mathrm{Y}$ if $\mathrm{A} = \mathrm{a}$

(b) The effect of the exposure A on outcome Y is unconfounded given C. There is no other unmeasured confounding of the A→Y relationship. The measured covariates $\{\mathrm{C}_1, \mathrm{C}_2, \mathrm{C}_6\}$ are in the data generating process for the outcome A and suffice to control for confounding for A-Y relationship. (Figure 1).

$$\mathrm{Y}^{\mathrm{a},\mathrm{m}_1,\mathrm{m}_2} \perp \mathrm{A} \,\big|\, \{\mathrm{C}_1, \mathrm{C}_2, \mathrm{C}_6\}$$

(c) The effect of M_1 and M_2 on outcome Y is unconfounded conditional on A and C**,** where $\mathrm{C} = \{\mathrm{C}_1, \mathrm{C}_2, \mathrm{C}_3, \mathrm{C}_4, \mathrm{C}_5, \mathrm{C}_6\}$ are covariates observed for the data generating process for $\{\mathrm{M}_1, \mathrm{M}_2\}$ and Y. $\{\mathrm{A}, \mathrm{C}_1, \mathrm{C}_4, \mathrm{C}_5\}$ are sufficient covariates for identifying the association between M_1 and Y. $\{\mathrm{A}, \mathrm{C}_3, \mathrm{C}_4, \mathrm{C}_6, \mathrm{M}_1\}$ are sufficient covariates for identifying the association between M_2 and Y.

$$\mathrm{Y}^{\mathrm{a},\mathrm{m}_1,\mathrm{m}_2} \perp \mathrm{M}_1^{\mathrm{a}'} \mid \{\mathrm{A}, \mathrm{C}_1, \mathrm{C}_4, \mathrm{C}_5\}$$

$$\mathrm{Y}^{\mathrm{a},\mathrm{m}_1,\mathrm{m}_2} \perp \mathrm{M}_2^{\mathrm{a}'} \mid \{\mathrm{A}, \mathrm{C}_3, \mathrm{C}_4, \mathrm{C}_6, \mathrm{M}_1\}$$

$$\mathrm{Y}^{\mathrm{a},\mathrm{m}_1,\mathrm{m}_2} \perp (\mathrm{M}_1^{\mathrm{a}'}, \mathrm{M}_2^{\mathrm{a}'}) \mid \{\mathrm{A}, \mathrm{C}_1, \mathrm{C}_3, \mathrm{C}_4, \mathrm{C}_5, \mathrm{C}_6\}$$

(d) The effect of A on both mediators is unconfounded conditional on C. There are no unobserved confounders between A and any of $\{\mathrm{M}_1, \mathrm{M}_2\}$, and $\{\mathrm{C}_1, \mathrm{C}_4, \mathrm{C}_6\}$ are sufficient to adjust for confounding of the effects of A on $\{M_1, M_2\}$.

$$\{M_1^a, M_2^a)\} \perp A \;\middle|\; \{C_1, C_4, C_6\}$$

$$M_1^a \perp A \;\middle|\; C_1$$

$$M_2^a \perp A \;\middle|\; \{C_1, C_4, C_6\}$$

CHAPTER 3. THE PROPOSED SIMULATION PROCEDURE

3.1 Super population

A study (9) reported that among 2289 adults aged 71 to 82 years at baseline, 31.76% had poor olfaction ($\mathrm{A} = 1$), 37.3% had neurodegenerative disease ($\mathrm{M}_1 = 1$), 19.1% had weight loss ($\mathrm{M}_2 = 1$), Mortality 52.91% ($\mathrm{Y} = 1$) by year 13. We created a super population (Table 1) with the prevalence of the exposure (A), mediators (M_1 and M_2), and outcome (Y) similar in the paper (9) as shown in table 1 based on the data generating process in Figure 1.

Table 2. Prevalence of Exposure, Mediators, and Outcome in Super-population

		N=10,000,000 Frequency (Percentage)
A	0	6,962,575 (69.6%)
	1	3,037,425 (30.4%)
M_1	0	6,525,293 (65.3%)
	1	3,474,707 (34.7%)
M_2	0	7,635,725 (76.4%)
	1	2,364,275 (23.6%)
Y	0	4,962,836 (49.6%)
	1	5,037,164 (50.4%)

3. 2 Equation for data generating procedure (DGP)

In this chapter, we provide details about our simulation study. We assumed all covariates are independent and identically distributed in our study. We generated six independent baseline covariates $\mathrm{C}_1 - \mathrm{C}_6$ with identical distribution: $\mathrm{Bernoulli}(1, 0.5)$.

$\mathrm{C_1, C_2, C_3, C_{4,} C_5, C_6} \sim \mathrm{Bernoulli}(1, 0.5)$

Next, we generated A, $\mathrm{M_1}$, $\mathrm{M_2}$ and Y with Bernoulli distribution as described below.

iid: A sequence of independent, identically distributed (IID) random variables

$$\mathrm{P(A)} = \frac{\exp[-\log(14) + \log(3)\,\mathrm{C_1} + \log(3)\,\mathrm{C_2} + \log(3)\,\mathrm{C_6}]}{1 + \exp[-\log(14) + \log(3)\,\mathrm{C_1} + \log(3)\,\mathrm{C_2} + \log(3)\mathrm{C_6}]}$$

$A \sim \mathrm{Bernoulli}(1, \mathrm{P(A)})$

$$\mathrm{P(M_1)} = \frac{\exp[-\log(16) + \log(3)\,\mathrm{C_1} + \log(3)\,\mathrm{C_4} + \log(3)\,\mathrm{C_5} + \log(3)\mathrm{A}]}{1 + \exp[-\log(16) + \log(3)\,\mathrm{C_1} + \log(3)\,\mathrm{C_4} + \log(3)\,\mathrm{C_5} + \log(3)\mathrm{A}]}$$

$M_1 \sim \mathrm{Bernoulli}(1, \mathrm{P(M_1)})$

$$\mathrm{P(M_2)} = \frac{\exp[-\log(50) + \log(3)\,\mathrm{C_3} + \log(3)\,\mathrm{C_4} + \log(3)\,\mathrm{C_6} + \log(3)\mathrm{A} + \log(2)\mathrm{M_1} + \log(2)\mathrm{AM_1}]}{1 + \exp[-\log(50) + \log(3)\,\mathrm{C_3} + \log(3)\,\mathrm{C_4} + \log(3)\,\mathrm{C_6} + \log(3)\mathrm{A} + \log(2)\mathrm{M_1} + \log(2)\mathrm{AM_1}]}$$

$\mathrm{M_2} \sim \mathrm{Bernoulli}(1, \mathrm{P(M_2)})$

$$\mathrm{P(Y)} = \frac{(\exp[-\log(3)\mathrm{C_2} + \log(3)\mathrm{C_3} + \log(3)\mathrm{C_5} + \log(3)\mathrm{M_1} + \log(3)\mathrm{M_2} + \log(4)\mathrm{A} + \log(4)\mathrm{M_1M_2} + \log(4)\mathrm{AM_1} + \log(4)\mathrm{AM_2}]}{1 + (\exp[-\log(3)\mathrm{C_2} + \log(3)\mathrm{C_3} + \log(3)\mathrm{C_5} + \log(3)\mathrm{M_1} + \log(3)\mathrm{M_2} + \log(4)\mathrm{A} + \log(4)\mathrm{M_1M_2} + \log(4)\mathrm{AM_1} + \log(4)\mathrm{AM_2}]}$$

$\mathrm{Y} \sim \mathrm{Bernoulli}(1, \mathrm{P(Y)})$

3.3 True effects

After generating the exposure, outcome, and mediator variables as mentioned in section 3.2 DGP, we further generated counterfactual outcomes and estimated true effects using the superpopulation (N=10,000,000) discussed in section 2.4c. The calculated true effects are listed in table 2.

Table 3. True causal effects

	Risk Difference (RD)				Risk Ratio (RR)			
True effects for:	$\mathrm{TE_{RD}}$	$\mathrm{NDE_{RD}}$	$\mathrm{NIE_{RD}}$	$\mathrm{PIE_{RD}}$	$\mathrm{TE_{RR}}$	$\mathrm{NDE_{RR}}$	$\mathrm{NIE_{RR}}$	$\mathrm{PIE_{RR}}$
Lange and Steen methods	0.404	0.297	0.065	0.043	2.052	1.773	1.089	1.063
Hong method			0.044	0.063			1.059	1.093

These true effects are one decomposition of the natural effects as discussed in section 2.3a. The total effect in risk difference scale ($\mathrm{TE_{RD}}$) is the sum of the component effects ($\mathrm{NDE_{RD}} + \mathrm{NIE_{RD}} + \mathrm{PIE_{RD}}$) and the total effect in risk ratio scale is the product of the component effects. The DGP as described in equations (section 3.2) was used for each simulation to generate 2000 observations. The direct, indirect, and total effects of interest were estimated within each scenario using the three statistical approaches discussed below. To assess performance, method- and scale-specific effects from each simulation were then compared to the true effects obtained from the super population (2, 3).

3.4 The proposed estimation procedure

The rationale for how the newly modified procedure featuring two causally ordered mediators provides validity is shown in Appendices A and B. We performed Monte-Carlo simulations similar to the Lange and Steen simulations, and extended them to Hong's method.

We carried out Monte Carlo simulation based on generalizations of Steen's mediation formula as shown in Figure 2 (3, 11). The Lange, Steen and Hong approaches share several similarities in their respective procedures (Figure 2). All methods require expansions of the original dataset, need to generate weights for at least one mediator and use those weights to fit a suitable model to the outcome variable. In addition, each approach used counterfactual-framework based on a marginal structural model (MSM) to estimate causal mediation effects (2, 3).

As shown in Figure 2, the three methods are different in the process of data simulation. Prior to data expansion and weight generation, the Lange and Hong methods require that both M_1 and M_2 to be modeled, while disregarding any model for Y. On the other hand, the Steen method needs one mediator (M_1 or M_2) and Y to be modeled. Additional differences were discussed as follows and in Figure 2.

Model specifications were different for three methods. We fitted a logistic regression model for binary mediators M_1, M_2 conditional on A and covariate set C.

$$\mathrm{logit}[\Pr(M_1 = 1)|A, \mathbf{C}] = \alpha_0 + \alpha_1 * A + \alpha_2^T * C \qquad (1)$$

$$\mathrm{logit}[\Pr(M_2 = 1)|A, M_1, \mathbf{C}\,] = \theta_0 + \theta_1 * A + \theta_2 * M_1 + \theta_3 * A * M_1 + \theta_4^T * \mathbf{C} \qquad (2)$$

Lange and Hong methods: both (1) and (2), but Steen method: either (1) or (2).

With regard to Steen method, fit a logistic regression model for the binary outcome

Y conditional on A, both $\mathrm{M_1}$ and $\mathrm{M_2}$, covariate set C as shows in equation (3).

$$\mathrm{logit}[\Pr(Y=1)|\mathrm{A},\mathrm{M_1},\mathrm{M_2},\mathbf{C}] = \gamma_0 + \gamma_1 * \mathrm{A} + \gamma_2 * \mathrm{M_1} + \gamma_3 * \mathrm{M_2} + \gamma_4 * \mathrm{A} * \mathrm{M_1} + \gamma_5 * \mathrm{A} * \mathrm{M_2}$$
$$+ \gamma_6 * \mathrm{M_1} * \mathrm{M_2} + \gamma_7 * \mathrm{A} * \mathrm{M_1} * \mathrm{M_2} + \gamma_8^{\mathrm{T}} * \mathrm{C} \qquad (3)$$

Three methods employ various weighting in MSM (1-3). The section below describes how the weights are generated.

Lange weight was given by $(\mathrm{i}=0{,}1, \mathrm{k}=1{,}2)$

$$\mathrm{W_i} = \frac{1}{\Pr(\mathrm{A}=\mathrm{A^i}|\mathrm{C}=\mathrm{c})} \prod_{\mathrm{k}=1}^{\mathrm{K}} \frac{\Pr\left(\mathrm{M_k}=\mathrm{M_k^i}\middle|\mathrm{A}=\mathrm{A_k^i}, \mathrm{C}=c\right)}{\Pr\left(\mathrm{M_k}=\mathrm{m_1}\middle|\mathrm{A}=\mathrm{A^i}, \mathrm{C}=\mathrm{c}\right)}$$
$$= \Pr(\mathrm{M_1}=\mathrm{m_1}|\mathrm{A}=\mathrm{a}, \mathrm{C}=\mathrm{c}) * \frac{\Pr(\mathrm{M_1}=\mathrm{m_1}|\mathrm{A}=\mathrm{a_1}, \mathrm{C}=\mathrm{c})}{\Pr(\mathrm{M_1}=\mathrm{m_1}|\mathrm{A}=\mathrm{a}, \mathrm{C}=\mathrm{c})}$$

$$* \Pr(\mathrm{M_2}=\mathrm{m_2}|\mathrm{A}=\mathrm{a}, \mathrm{C}=\mathrm{c}) * \frac{\Pr(\mathrm{M_2}=\mathrm{m_2}|\mathrm{A}=\mathrm{a_2}, \mathrm{C}=\mathrm{c})}{\Pr(\mathrm{M_2}=\mathrm{m_2}|\mathrm{A}=\mathrm{a}, \mathrm{C}=\mathrm{c})} * \frac{1}{\Pr(\mathrm{A}=\mathrm{a}|\mathrm{C}=\mathrm{c})}$$

Steen weight was computed by $(\mathrm{i}=0{,}1, \mathrm{k}=1{,}2)$

$$\mathrm{W_{1i,a'}} = \frac{\Pr\left(\mathrm{M_1}=\mathrm{M_1^i}\middle|\mathrm{A}=\mathrm{a'}, \mathrm{C}=\mathrm{C^i}\right)}{\Pr\left(\mathrm{M_1}=\mathrm{M_1^i}\middle|\mathrm{A}=\mathrm{a''}, \mathrm{C}=\mathrm{C^i}\right)} = \frac{\Pr\left(\mathrm{M_1}=\mathrm{M_1^i}\middle|\mathrm{A}=\mathrm{a'}, \mathrm{C}=\mathrm{C^i}\right)}{\Pr\left(\mathrm{M_1}=\mathrm{M_1^i}\middle|\mathrm{A}=\mathrm{A^i}, \mathrm{C}=\mathrm{C^i}\right)}$$

$$\mathrm{W_{2i,a''}} = \frac{\Pr\left(\mathrm{M_2}=\mathrm{M_2^i}\middle|\mathrm{A}=\mathrm{a''}, \mathrm{C}=\mathrm{C^i}\right)}{\Pr\left(\mathrm{M_2}=\mathrm{M_2^i}\middle|\mathrm{A}=\mathrm{a'}, \mathrm{C}=\mathrm{C^i}\right)} = \frac{\Pr\left(\mathrm{M_2}=\mathrm{M_2^i}\middle|\mathrm{A}=\mathrm{a''}, \mathrm{C}=\mathrm{C^i}\right)}{\Pr\left(\mathrm{M_2}=\mathrm{M_2^i}\middle|\mathrm{A}=\mathrm{a''}, \mathrm{C}=\mathrm{C^i}\right)}$$
, where C is a set of confounders.

Hong weights were computed as shown in Table 3.

Table 4. Hong weights for marginal structural models.

	A	a'	a''	D	Weight
$E(Y^{0, M_1^0, M_2^{0, M_1^0}})$	0	0	0	0	1.0
$E(Y^{1, M_1^0, M_2^{0, M_1^0}})$	1	0	0	0	$\frac{\theta_{M_1^0=m_1}}{\theta_{M_1^1=m_1}} * \frac{\theta_{M_2^{0,m_1}=m_2}}{\theta_{M_2^{1,m_1}=m_2}} = \frac{\Pr(M_1^0 = m_1 \| A = 0, C = c)}{\Pr(M_1^1 = m_1 \| A = 1, C = c)} * \frac{\Pr(M_2^{0,M_1} = m_2 \| A = 0, M_1^0 = m_1, C = c)}{\Pr(M_2^{1,M_1} = m_2 \| A = 1, M_1^1 = m_1, C = c)}$
$E(Y^{1, M_1^1, M_2^{0, M_1^0}})$	1	1	0	0	$\frac{\theta_{M_2^{0,m_1}=m_2}}{\theta_{M_2^{1,m_1}=m_2}} = \frac{\Pr(M_2^{0,M_1} = m_2 \| A = 0, M_1^0 = m_1, C = c)}{\Pr(M_2^{1,M_1} = m_2 \| A = 1, M_1^1 = m_1, C = c)}$
$E(Y^{1, M_1^0, M_2^{1, M_1^1}})$	1	0	1	0	$\frac{\theta_{M_1^0=m_1}}{\theta_{M_1^1=m_1}} = \frac{\Pr(M_1^0 = m_1 \| A = 0, C = c)}{\Pr(M_1^1 = m_1 \| A = 1, C = c)}$
$E(Y^{1, M_1^1, M_2^{1, M_1^1}})$	1	1	0	1	1.0

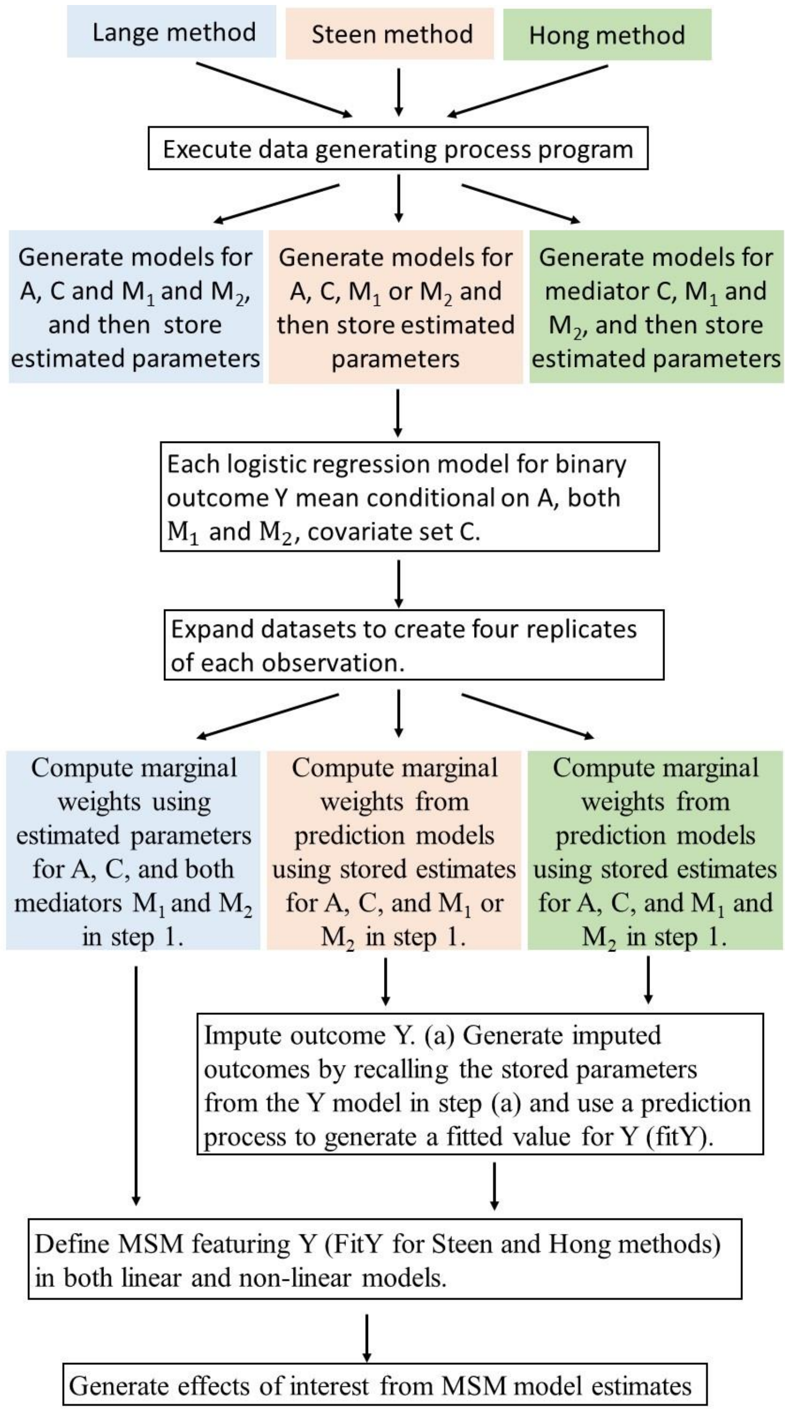

Figure 2. Chart of the procedures executed for each mediation analysis method

CHAPTER 4. DATA SIMULATION

We compared three estimation approaches for sequential mediation analysis including their software implementations in STATA version 16 (StataCorp LP, College Station, Texas).

In this section we present the simulation scenarios designed to compare the robustness of three approaches in estimating causal effects, as well as the criteria used to compare the performance of the three methods.

4.1 Simulation Scenarios

In addressing causal effects, we control for variables that are confounders ($C_1 - C_6$) of the A→Y relationship (C_1, C_2, C_6), of the A→M_1 relationship (C_1), of the A→M_2 relationship (C_1, C_6), M_1→M_2 relationship (A, C_4, C_6) of M_1→Y relationship (A, C_1, C_4, C_5) and of $M_2 - Y$ relationship (A, C_2, C_3, C_5, M_1) (Section 2.2). We selected N = 2000 representing a relatively large sample size. We designed a range of scenarios that mis-specify the aforementioned relationships based on the causal pathway (Figure 1) so that we can understand the robustness of the three methods under even unusual conditions.

In total we evaluated the correctly-specified model (CSM) and 12 scenarios for Lange and Hong methods, two CSM (CSM1 and CSM2) and 22 scenarios for Steen method (Table 5). The CSM represents the case in which all models were correctly specified. Scenarios 1-9 are shared by all three methods. The first four scenarios represent cases in which M_1 is mis-specified when C_1 (scenario 1, omitting confounding variable for A→M_1 relationship), or C_4 (scenario 2, omitting unnecessary covariate) or C_5 (scenario 3, omitting unnecessary covariate), or $(C_1 + C_4 + C_5)$ (scenario 4) were omitted from the variable generation equation. Scenarios 5-8 represent cases in which M_2 was mis-specified when C_3 (scenario 5, omitting unnecessary covariate), or C_4 (scenario 6, omitting confounding variable for $M_1 \rightarrow M_2$ relationship) or C_6 (scenario 7, omitting confounding variable for $A \rightarrow M_2$ relationship), or $(C_3 + C_4 + C_6)$ (scenario 8) were omitted from the variable generation equation. Scenario 9 portrays the case in which M_2 is mis-specified due to $A * M_1$ interaction being omitted. For Lange and Hong methods, scenarios 10-12 represent cases in which both M_1 and M_2 were mis-specified due to unmeasured confounding as shown in Table 5.

With regard to Steen method, CSM1 and CSM2 are both the correctly-specified models, CSM1 is the model when M_1 and Y are included to generated IPTW, and CSM2 is the model when M_2 and Y are included to generated IPTW (Table 5). Scenarios 10-13 were cases in which Y was mis-specified due to unmeasured confounding (Table 5). Scenarios 14-16 represented mis-specified cases in which $A * M_1$ interaction (scenario 14), $A * M_2$ interaction (scenario 15), or $M_1 * M_2$ interaction (scenario 16) were omitted. Scenarios 17-19 were cases in which both M_1 and Y were mis-specified due to unmeasured confounding; and scenarios 20-22 represented cases in which both M_2 and Y are mis-specified due to unmeasured confounding.

We discussed the confounder for each relation in section 2.2. Under the assumption that no-omitted confounders in our DAG (Figure 1), omitting C_1 in scenarios 1, 4, 10 or 17 would increase bias for NIE due to the fact C_1 is the confounder of $A * M_1$ relation (Table

5). On the other hand, omitting C_4 in scenarios 6, 8, 11, or 21 would increase bias for PIE due to the fact C_4 is the confounder of $M_1 \rightarrow M_2$ relation (Table 5). When $A * M_1$ interaction is omitted in scenario 9, bias for PIE would rise compared to CSM. Omitting C_3 in scenarios 11 or 21 would increase bias for PIE due to the fact C_3 is the confounder of $M_2 \rightarrow Y$ relation. In addition, when $A * M_1$ interaction is omitted in scenario 14, bias for NIE would increase; when $A * M_2$ interaction is omitted in scenario 15, bias for PIE would increase. Lastly, when C_2 is omitted in scenarios 13, 17 or 20, bias for NDE would increase. We would provide performance criteria that would be used to compare three mediation analysis methods from different simulation scenarios for illustration (section 4.2).

Each scenario varies one feature of the data-generating process at a time. The changes of the parameter values in the inverse-probability-of-treatment weighting (IPTW) or ratio-of-mediator-probability weighting (RMPW) models lead to changes in IPTW or RMPW weights and correspondingly the performance of the estimators in the section 4.2. In this way, we can evaluate the influence of each data generation feature on the estimation results and assess the stability of performance for each estimation procedure. Equations used to generate M_1, M_1 and Y are as follows:

$$\text{logit}[\Pr(M_1 = 1)|A, C_1, C_4, C_5] = \alpha_0 + \alpha_1 * A + \alpha_2 * C_1 + \alpha_3 * C_4 + \alpha_4 * C_5 \quad (4.1)$$

$$\text{logit}[\Pr(M_2 = 1)|A, M_1, C_3, C_4, C_6] = \beta_0 + \beta_1 * A + \beta_2 * C_3 + \beta_3 * C_4 + \beta_4 * C_6 + \beta_5 * M_1 + \beta_6 * A * M_1 \quad (4.2)$$

$$\text{logit}[\Pr(Y = 1)|A, M_1, M_2, C_2, C_3, C_5] = r_0 + r_1 * A + \beta_2 * C_2 + r_3 * C_3 + r_4 * C_5 + r_5 * M_1 + r_6 * M_2 + r_7 * A * M_1 + r_8 * A * M_2 + r_9 * A * M_1 * M_2 \quad (4.3)$$

Table 5. Methods and simulation scenarios

Method	Scenario	Description	Explanation	Expectation of bias in theory
Lange Hong Steen	CSM	Model for M_1: Equation (4.1) Model for M_2: Equation (4.2)	CSM=Correctly-specified model	No
	1	C_1 is omitted from model (4.1)	M_1 is misspecified due to unmeasured confounding	Yes in NIE
	2	C_4 is omitted from model (4.1)		No
	3	C_5 is omitted from model (4.1)		No
	4	C_1, C_4, C_5 are omitted from model (4.1)		Yes in NIE
	5	C_3 is omitted from model (4.2)	M_2 is misspecified due to unmeasured confounding	No
	6	C_4 is omitted from model (4.2)		Yes in PIE
	7	C_6 is omitted from model (4.2)		No
	8	C_3, C_4, C_6 are omitted from model (4.2)		Yes in PIE
	9	$A * M_1$ is omitted from model (4.2)	M_2 is misspecified due to lack of interaction	Yes in PIE
Lange Hong	10a	C_1 is omitted from model (4.1) and C_3 is omitted from model (4.2)	Both M1 and M2 are misspecified due to unmeasured confounding	Yes in NIE
	11a	C_4 is omitted from model (4.1) and C_4 is omitted from model (4.2)		Yes in NIE, PIE
	12a	C_5 is omitted from model (4.1) and C_6 is omitted from model (4.2)		No
	CSM1	Model for M_1: Equation (4.1) Model for Y: Equation (4.3)	CSM1=Correctly-specified model 1	No

Table 5 (cont'd)

Steen	CSM2	Model for M_2: Equation (4.2) Model for Y: Equation (4.3)	CSM2=Correctly-specified model 2	No
	10b	C_2 is omitted from model (4.3)	Y is misspecified due to unmeasured confounding	Yes in NDE
	11b	C_3 is omitted from model (4.3)		Yes in PIE
	12b	C_5 is omitted from model (4.3)		Yes in NIE
	13	C_2, C_3, C_5 are omitted from model (4.3)		Yes in all
	14	$A * M_1$ is omitted from model (4.3)	Y is misspecified due to lack of interaction	Yes in NIE
	15	$A * M_2$ is omitted from model (4.3)		Yes in PIE
	16	$M_1 * M_2$ is omitted from model (4.3)		Yes in NIE
	17	C_1 is omitted from model (4.1) and C_2 is omitted from model (4.3)	M_1 and Y are misspecified due to unmeasured confounding	Yes in NDE, NIE
	18	C_4 is omitted from model (4.1) and C_3 is omitted from model (4.3)		Yes in PIE
	19	C_5 is omitted from model (4.1) and C_5 is omitted from model (4.3)		No
	20	C_3 is omitted from model (4.2) and C_2 is omitted from model (4.3)	M_2 and Y are misspecified due to unmeasured confounding	Yes in NDE
	21	C_4 is omitted from model (4.2) and C_3 is omitted from model (4.3)		Yes in PIE
	22	C_6 is omitted from model (4.2) and C_5 is omitted from model (4.3)		No

4.2 Performance criteria

The causal mediation estimation was replicated 2000 times for each scenario. We used three criteria to examine the performance of three estimation methods (3, 19):

(1) Bias was computed by subtracting the true value of the parameter from the parameter estimate:

$$\text{Bias} = \frac{1}{2000} * \sum_{i=1}^{2000} (\hat{\theta}_i - \theta_{tr})$$

$\hat{\theta}_i$ is the causal mediation effect estimate for the i^{th} simulation.

(2) Root mean square error (RMSE): RMSE was computed by subtracting the true value of the parameter from the parameter estimate, squaring this value, and then adding the empirical variance of the parameter estimate. Low values of RMSE reflect either low bias, high precision, or some combination of the two.

$$\text{RMSE} = \sqrt{\frac{1}{2000} * \sum_{i=1}^{2000} (\hat{\theta}_i - \theta_{tr})^2}$$

(3) Confidence interval coverage: We calculated the probability of true estimates falling into actual 95% confidence interval (CI) across the 2,000 replications.

$$\text{Coverage} = \frac{1}{2000} * \sum_{i=1}^{2000} [\theta_{tr} \in (LB_i, UB_i)]$$

where LB_i is lower bound, UB_i is upper bound of the corresponding effect for the i^{th} simulation.

CHAPTER 5. SIMULATION RESULTS

To investigate the robustness of the considered estimation approaches, we performed simulation studies with 2000 runs of data sets with 2000 observations. Each method estimates total effect (TE), natural direct effect (NIE), natural indirect effect (NIE) and partial indirect effect (PIE) as they differ in how the weights are generated and how they handle confounders and interactions. To make the simulation results more comparable, we reported the bias, root mean squared error (RMSE), and coverage probability of the 95% confidence interval (CI) across 2,000 simulations relative to the true causal effects estimated from a large simulated data set (Table 6), as discussed in section 3.3.

Low values (or absolute values) of bias indicate low bias. Low values (or absolute values) of RMSE reflect low bias, high precision, or the combination of the two. High values of coverage probability (The closer to 95%, the better.) indicate better performance.

Table 6. Performance in risk difference and risk ratio scales when the all parameters were correctly specified in the three methods.

True effect	Metric	**Lange** CSM	**Steen** CSM1	**Steen** CSM2	**Hong** CSM
$\mathrm{TE}_{\mathrm{RD}} = 0.404$	Bias	0.004 (0.017)	0.004 (0.024)	0.004 (0.024)	0.000 (0.024)
	RMSE	0.611 (0.455)	0.847 (0.705)	0.850 (0.705)	0.832 (0.700)
	Coverage	94.3%	80.6%	81.3%	82.3%
$\mathrm{NDE}_{\mathrm{RD}} = 0.297$	Bias	0.005 (0.020)	0.005 (0.024)	0.005 (0.024)	0.001 (0.028)
	RMSE	0.740 (0.555)	0.876 (0.656)	0.878 (0.656)	0.984 (0.744)
	Coverage	95.7%	25.3%	26.8%	77.3%
$\mathrm{NIE}_{\mathrm{RD}} = 0.065$ $\mathrm{NIE}_{\mathrm{RD}}^{\mathrm{Hong}} = 0.044$	Bias	0.003 (0.007)	0.003 (0.011)	0.003 (0.016)	0.022 (0.010)
	RMSE	0.266 (0.210)	0.388 (0.301)	0.575 (0.435)	0.969 (0.446)
	Coverage	88.0%	62.3%	95.2%	7.3%
$\mathrm{PIE}_{\mathrm{RD}} = 0.043$ $\mathrm{PIE}_{\mathrm{RD}}^{\mathrm{Hong}} = 0.063$	Bias	-0.003 (0.006)	-0.004 (0.017)	-0.004 (0.010)	-0.022 (0.010)
	RMSE	0.253 (0.188)	0.610 (0.501)	0.360 (0.337)	1.005 (0.447)
	Coverage	83.7%	96.0%	66.5%	11.2%
$\mathrm{TE}_{\mathrm{RR}} = 2.052$	Bias	0.021 (0.060)	0.020 (0.090)	0.020 (0.090)	0.004 (0.088)
	RMSE	2.273 (1.722)	3.167 (2.616)	3.171 (2.622)	3.040 (2.498)
	Coverage	94.3%	76.8%	77.8%	78.0%
$\mathrm{NDE}_{\mathrm{RR}} = 1.773$	Bias	0.020 (0.062)	0.020 (0.079)	0.020 (0.079)	0.005 (0.086)
	RMSE	2.329 (1.778)	2.899 (2.223)	2.907 (2.222)	3.065 (2.354)
	Coverage	95.5%	40.1%	41.5%	77.3%
$\mathrm{NIE}_{\mathrm{RR}} = 1.089$ $\mathrm{NIE}_{\mathrm{RR}}^{\mathrm{Hong}} = 1.059$	Bias	0.004 (0.011)	0.005 (0.017)	0.005 (0.023)	0.032 (0.016)
	RMSE	0.423 (0.339)	0.614 (0.493)	0.848 (0.656)	1.440 (0.712)
	Coverage	90.5%	65.0%	95.0%	9.5%
$\mathrm{PIE}_{\mathrm{RR}} = 1.063$ $\mathrm{PIE}_{\mathrm{RR}}^{\mathrm{Hong}} = 1.093$	Bias	-0.005 (0.010)	-0.006 (0.026)	-0.006 (0.016)	-0.033 (0.016)
	RMSE	0.395 (0.291)	0.918 (0.754)	0.562 (0.516)	1.474 (0.696)
	Coverage	85.0%	95.7%	64.3%	15.1%

Note: Values in parentheses are the standard deviations (sd) of the measure of interest from 2000 simulations.
Abbreviations: NDE=Natural direct effect, NIE=Natural indirect effect, PIE=Partial indirect effect, TE=Total effect, RD=Risk difference, GLM=Generalized linear model, RMSE=Root mean square error.

5.1 Comparison across scenarios within each method

5.1a Simulation results generated from Lange method

In Table 6, each row represents a specific causal effect in RD or RR scale. The robustness of each method in accessing causal effects was evaluated using bias, RMSE and coverage. For Lange and approach (Figure 3 and Table 6), when all parameters were correctly specified, the bias and RMSE in RD and RR scales for TE, NDE, NIE and PIE were small. The coverage in RD and RR scales were close to 95%, which indicates high precision in simulation (Table 6). However, the coverage in RD and RR scales for the NIE and PIE in RD and RR scales were lower than expected (≤90%) when all parameters were correctly specified.

Figure 3 shows the performance in bias, RMSE and coverage of Lange method in RD and RR scales across all scenarios. Table 7 displays the summary of consistency of Lange method in terms of with our expectation as mentioned in section 4.1. Across all scenarios, the coverage for TE and NDE in RD and RR scales were around 95% resilient to parametermis-specification, but the coverage for NIE and NDE in RD and RR scales were below 90% (Figure 3).

When M_1 was mis-specified due to omission of C_1, bias and RMSE for NIE in RD and RR scales increased, and coverage in RD and RR scales decreased in scenarios 1, 4 and 10 compared to those in CSM (Figure 3 and Table 7). C_1 is the confounder between $\mathrm{A} \rightarrow \mathrm{M}_1$ (Figure 1), C_4 and C_5 were included in the equation to generate M_1, but are not confounders between $\mathrm{A} \rightarrow \mathrm{M}_1$, therefore the larger bias for NIE occurred when C_1 was deleted in scenarios 1, 4 and 10. This result is consistent with our expectation of bias in theory (Table 5).

C_4 is the confounder between $\mathrm{M}_1 \rightarrow \mathrm{M}_2$ (Figure 1). When M_2 was mis-specified due to the omission of C_4, bias and RMSE for PIE in RD and RR scales increased in scenarios 6, 8 and 11 compared to those in CSM (Figure 3 and Table 7). The bias for NIE and PIE increased in scenario 11 when C_4 was omitted from both M_1 and M_2 models, which is consistent with our expectation of bias in theory (Table 5 and Table 7).

C_5 is the confounder between $\mathrm{M}_1 \rightarrow Y$. When C_5 was deleted from the equation to generate M_1, bias and RMSE for NIE in RD and RR scales increased and coverage decreased in scenarios 3 and 12. This result was against our expectation of bias in theory.

In summary, Lange approach performed well as expected with a few exceptions across all scenarios.

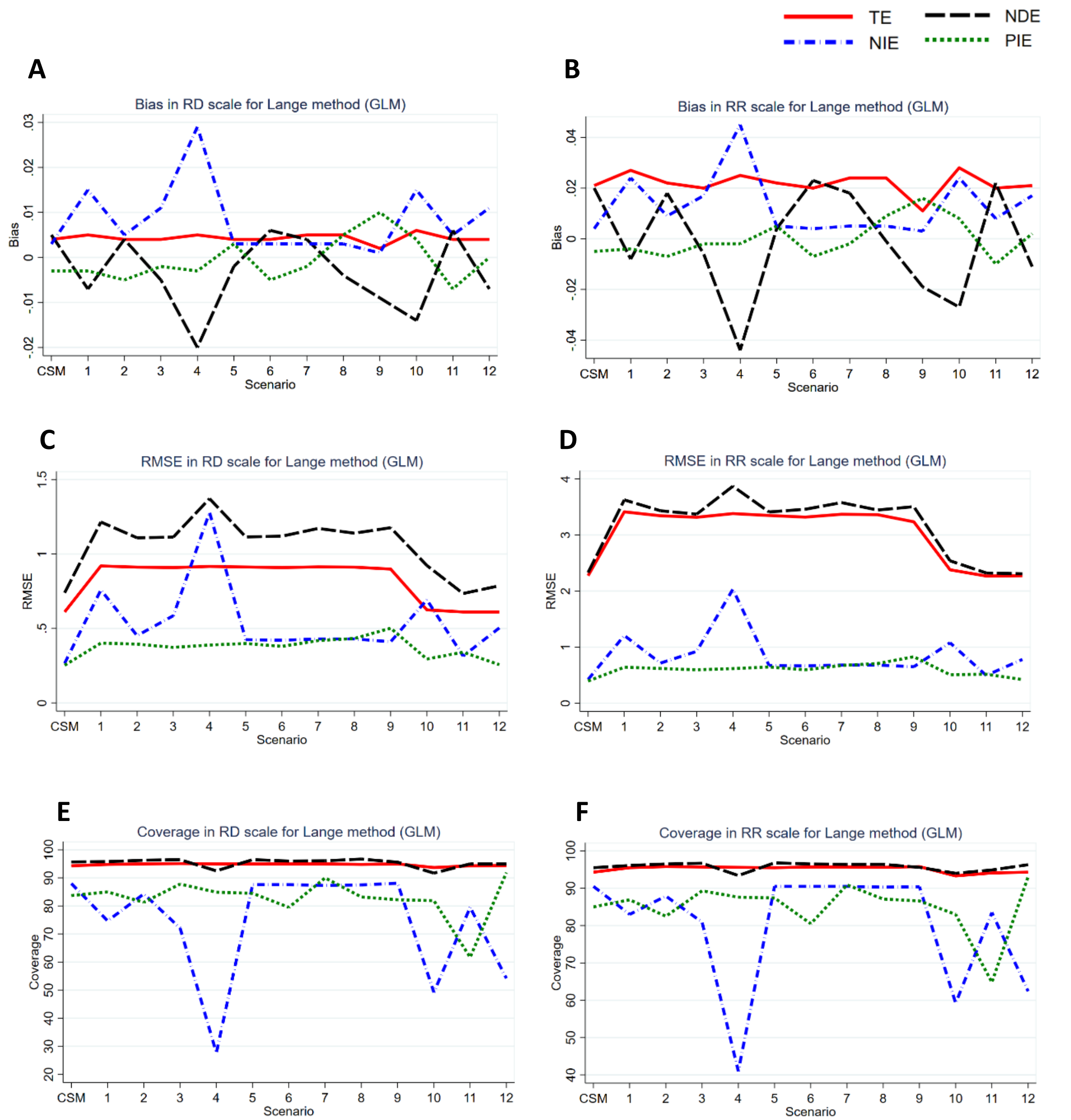

Figure 3. Performance of Lange method in risk difference (RD) scale and risk ratio (RR) scale

Solid red line represents TE; black long-dashed line represents NDE; blue dot-dashed line represents NIE; green short-dashed line represents PIE.

Bias in RD scale (A) and in RR scale (B); RMSE in RD scale (C) and RR scale (D); coverage probability in RD scale (E) and RR scale (F).

Table 7 Summary of Lange method in RD scale

Lange Scenario	Description	Expectation of bias in theory	Bias	RMSE	Coverage
CSM	CSM	No	No	No	No
1	$M_1 - C_1$	Yes in NIE	Yes in NIE	Yes in NIE	Yes in NIE
2	$M_1 - C_4$	No	No	No	No
3	$M_1 - C_5$	No	Yes in NIE	Yes in NIE	Yes in NIE
4	$M_1 - \{C_1, C_4, C_5\}$	Yes in NIE	Yes in NIE	Yes in NIE	Yes in NIE
5	$M_2 - C_3$	No	No	No	No
6	$M_2 - C_4$	Yes in PIE	No	Yes in PIE	Yes in PIE
7	$M_2 - C_6$	No	No	No	No
8	$M_2 - \{C_3, C_4, C_6\}$	Yes in PIE	Yes in PIE	Yes in PIE	No
9	$M_2 - \{A * M_1\}$	Yes in PIE	Yes in PIE	Yes in PIE	Yes in PIE
10a	$\{M_1 - C_1\}$ & $\{M_2 - C_3\}$	Yes in NIE	Yes in NIE	Yes in NIE	Yes in NIE
11a	$\{M_1 - C_4\}$ & $\{M_2 - C_4\}$	Yes in NIE, PIE	Yes in NIE, PIE	Yes in NIE, PIE	Yes in NIE, PIE
12a	$\{M_1 - C_5\}$ & $\{M_2 - C_6\}$	No	Yes in NIE	Yes in NIE	Yes in NIE

Note: Description column represents CSM or mis-specified models, for example, $M_1 - C_1$ indicates C_1 was omitted from M_1 equation.

5.2b Simulation results for Steen method

For Steen approach (Figure 4 and Table 6), when all parameters were correctly specified, the bias and RMSE in RD scales for TE, NDE, NIE and PIE were small as expected (Table 6), however the coverage in RD and RR scales for TE and NDE were much smaller than 95% (Table 6). The coverage in RD and RR scales for NIE were 95.2% and 95.0% in CSM2, in contrast, the coverage in RD and RR scales for PIE in CSM1 were poor. The coverage in RD and RR scales for PIE were 96.0% and 95.7% in CSM1, in contrast, the coverage in RD and RR scales for PIE in CSM2 are much smaller than 95%. Steen method showed different performance in coverage depending on whether M_1 or M_2 was used for weighting.

Figure 4 shows the performance in bias, RMSE and coverage of Steen method in RD and RR scales across all scenarios. Table 8 displays the summary of consistency of Steen method in terms of bias with our expectation as mentioned in section 4.1. Notably, across all scenarios, the coverage for TE and NDE in RD and RR scales was poor (<80%), furthermore, the coverage for NIE and PIE was below 90% in most scenarios (Figure 4).

C_1 is the confounder between $\mathrm{A} \rightarrow \mathrm{M}_1$ (Figure 1). When M_1 was mis-specified due to omission of C_1, the bias and RMSE for NIE in RD and RR scales increased in scenarios 1, 4 and 17 compared to those in CSM1 (Figure 4 and Table 8). Interestingly, the bias and RMSE for PIE in RD and RR scales were also increased in scenario 1 and 4.

C_4 is the confounder between $\mathrm{M}_1 \rightarrow \mathrm{M}_2$ (Figure 1). When M_2 was mis-specified due to the omission of C_4, bias and RMSE for PIE in RD and RR scales increased in scenarios 6 and 8 compared to those in CSM2 (Figure 4 and Table 8). The bias and RMSE for PIE did not increase as expected. Notably, the bias and RMSE for NIE increased in scenarios 6 and 8.

C_2 is the confounder between $A \rightarrow Y$. When C_2 was deleted from the equation to generate Y, the bias and RMSE for NDE in RD and RR scales increased in scenarios 10, 13, 17 and 20 as expected (Figure 4 and Table 8).

Deletion of $A * M_1$ interaction from Y model (scenario 14) led to bias and RMSE increase for NIE as expected (Figure 4 and Table 8). In addition, when $A * M_2$ interaction was omitted from Y model (scenario 15), the bias and RMSE for PIE increased as expected. When $M_1 * M_2$ interaction was omitted from Y model (scenario 16), the bias and RMSE for NIE did not change as expected (Figure 4 and Table 8).

There are two notable points to mention for Steen methods. First, in most scenarios when M_1 or M_2 was mis-specified, the bias and RMSE for NIE and NDE did not change as expected (Figure 4 and Table 6). Second, in scenarios 3, 7, 19 and 22, there were unexpected change in bias and RMSE (Table 6).

In summary, Steen approach seems less resilient to parameter mis-specification compared to Lange method in many scenarios. We would have a further comparison across the methods in the following sections.

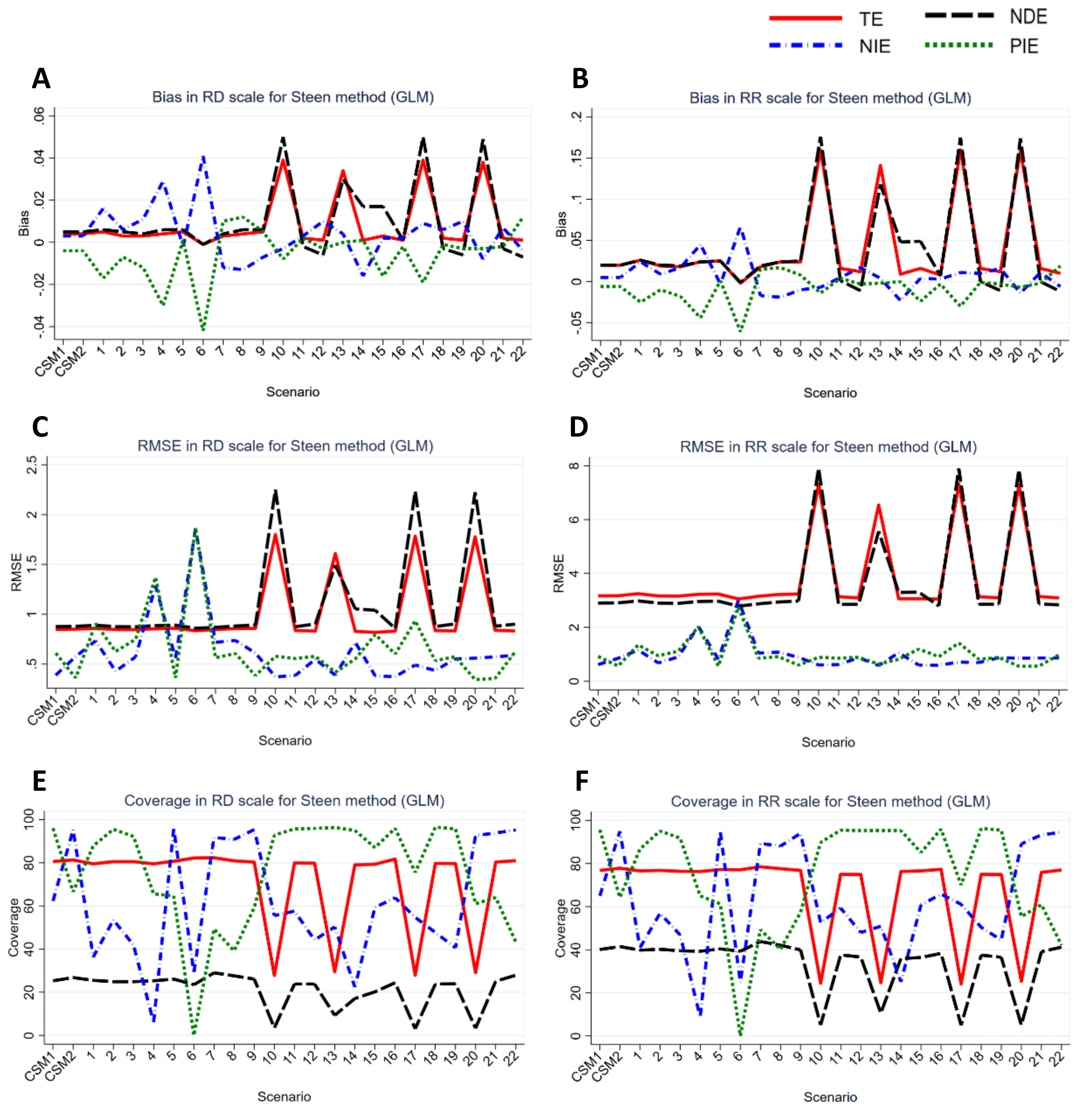

Figure 4. Performance of Steen method in risk difference (RD) scale or risk ratio (RR) scale

Solid red line represents TE; black long-dashed line represents NDE; blue dot-dashed line represents NIE; green short-dashed line represents PIE.

Bias in RD scale (A) and in RR scale (B); RMSE in RD scale (C) and RR scale (D); coverage probability in RD scale (E) and RR scale (F).

Table 8 Summary of Steen method in RD scale

Steen Scenario	Description	Expectation of bias in theory	Bias	RMSE
CSM1	CSM1	No	No	No
CSM2	CSM2	No	No	No
1	$M_1 - C_1$	Yes in NIE	Yes in NIE, PIE	Yes in NIE, PIE
2	$M_1 - C_4$	No	Yes in NIE, PIE	No
3	$M_1 - C_5$	No	Yes in NIE, PIE	Yes in NIE, PIE
4	$M_1 - \{C_1, C_4, C_5\}$	Yes in NIE	Yes in NIE, PIE	Yes in NIE, PIE
5	$M_2 - C_3$	No	No	No
6	$M_2 - C_4$	Yes in PIE	Yes in NIE, PIE	Yes in NIE, PIE
7	$M_2 - C_6$	No	Yes in NIE, PIE	Yes in NIE
8	$M_2 - \{C_3, C_4, C_6\}$	Yes in PIE	Yes in NIE, PIE	Yes in NIE
9	$M_2 - \{A * M_1\}$	Yes in PIE	Yes in NIE	Yes in NIE
10b	$Y - C_2$	Yes in NDE	Yes in NDE	Yes in NDE
11b	$Y - C_3$	Yes in PIE	No	No
12b	$Y - C_5$	Yes in NIE	Yes in NIE	Yes in NIE
13	$Y - \{C_2, C_3, C_5\}$	Yes in all	Yes in NDE	Yes in NDE
14	$Y - \{A * M_1\}$	Yes in NIE	Yes in NIE	Yes in NIE
15	$Y - \{A * M_2\}$	Yes in PIE	Yes in PIE	Yes in PIE
16	$Y - \{M_1 * M_2\}$	Yes in NIE	No	No
17	$\{M_1 - C_1\}$ & $\{Y - C_2\}$	Yes in NDE, NIE	Yes in NDE, NIE, PIE	Yes in NDE, NIE, PIE
18	$\{M_1 - C_4\}$ & $\{Y - C_3\}$	Yes in PIE	Yes in NIE	Yes in NIE
19	$\{M_1 - C_5\}$ & $\{Y - C_5\}$	No	Yes in NIE	Yes in NIE
20	$\{M_2 - C_3\}$ & $\{Y - C_2\}$	Yes in NDE	Yes in NDE, NIE	Yes in NDE, NIE
21	$\{M_2 - C_4\}$ & $\{Y - C_3\}$	Yes in PIE	Yes in NIE	Yes in NIE
22	$\{M_2 - C_6\}$ & $\{Y - C_5\}$	No	Yes in PIE	Yes in NIE

Note: Description column represents CSM1, CSM2 or mis-specified models, for example,

$M_1 - C_1$ indicates C_1 was omitted from M_1 equation.

5.1c Simulation results for Hong method

As shown in Table 6, for Hong method when all parameters were correctly-specified, bias and RMSE in TE and NDE in both RD and RR scales were smaller than those from Lange method and Steen method. On the other hand, Hong method had larger bias and RMSE for NIE and PIE in RD and RR scales (Table 6). The coverage from Hong method was poor, especially the coverage for NIE and PIE were extremely low (<20%).

Figure 5 shows the performance in bias, RMSE and coverage of Hong method in RD and RR scales across all scenarios. Table 9 displays the summary of consistency of Hong method in terms of with our expectation as mentioned in section 4.1. Across all scenarios, TE was resilient to parameter mis-specification (Figure 5). The coverage for TE, NDE, NIE and PIE in RD and RR scales were poor (<85%) in all scenarios (Figure 5).

When M_1 was mis-specified due to omission of C_1, bias and RMSE for NIE in RD and RR scales increased in scenarios 1, 4 and 10 compared to those in CSM (Figure 5 and Table 9). When M_2 was mis-specified due to the omission of C_4, bias and RMSE for PIE in RD and RR scales increased in scenarios 6, 8 and 11 compared to those in CSM (Figure 5 and Table 9). Surprisingly, when C_5 was deleted from the equation to generate M_1, bias and RMSE for NIE in RD and RR scales increased and coverage decreased in scenarios 3 and 12.

In summary, Hong method had higher bias and RMSE for NIE and PIE in RD and RR scales across all scenarios relative to the other two methods (Figure 5). In many scenarios, Hong approach showed bias and RMSE change as expected.

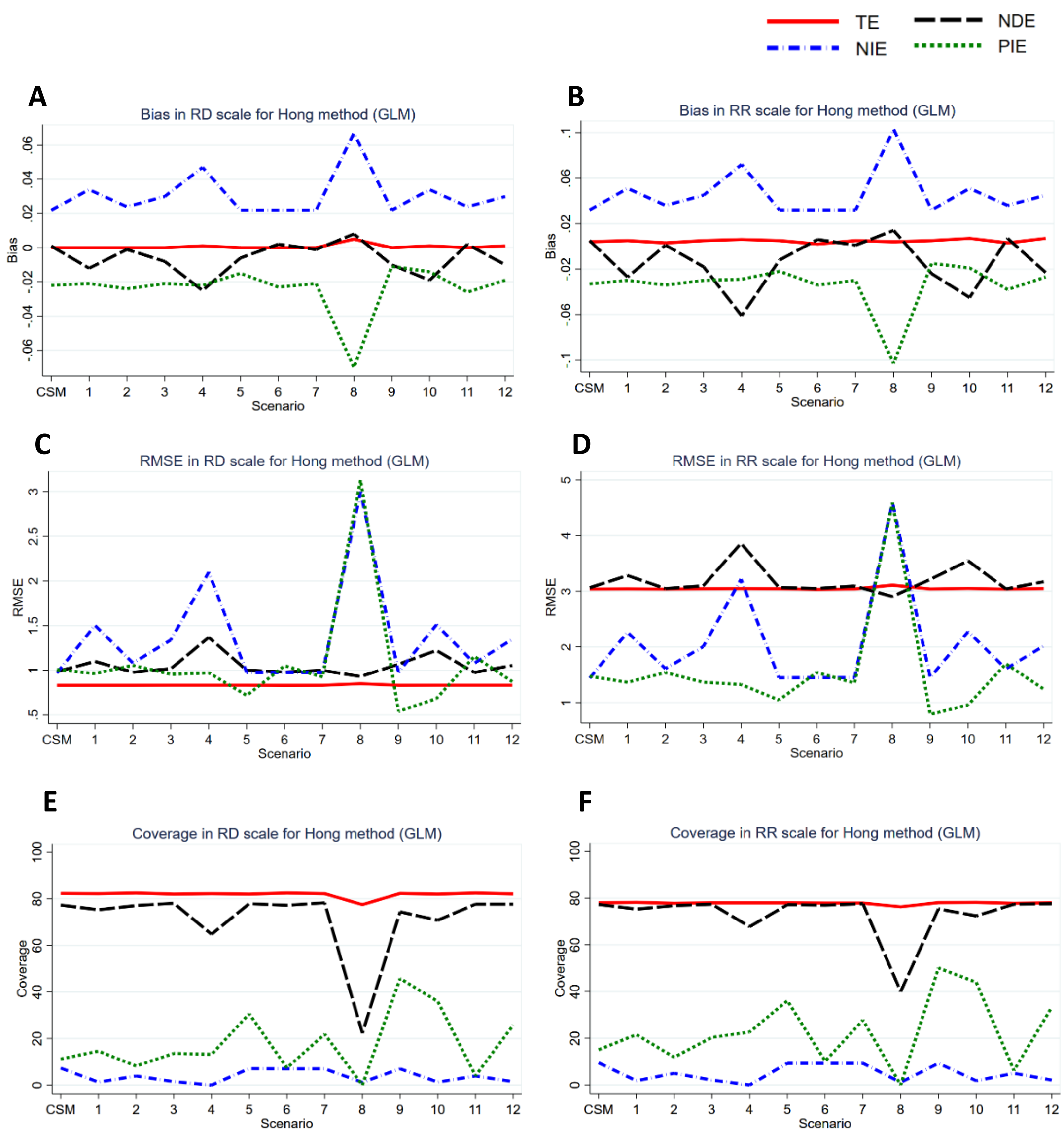

Figure 5. Performance of Hong method in risk difference (RD) scale or risk ratio (RR) scale

Solid red line represents TE; black long-dashed line represents NDE; blue dot-dashed line represents NIE; green short-dashed line represents PIE.

Bias in RD scale (A) and in RR scale (B); RMSE in RD scale (C) and RR scale (D); coverage probability in RD scale (E) and RR scale (F).

Table 9 Summary of Hong method in RD scale

Hong Scenario	Description	Expectation of bias in theory	Bias	RMSE
CSM	CSM	No	No	No
1	$M_1 - C_1$	Yes in NIE	Yes in NIE	Yes in NIE
2	$M_1 - C_4$	No	No	No
3	$M_1 - C_5$	No	Yes in NIE	Yes in NIE
4	$M_1 - \{C_1, C_4, C_5\}$	Yes in NIE	Yes in NIE	Yes in NIE
5	$M_2 - C_3$	No	No	No
6	$M_2 - C_4$	Yes in PIE	No	Yes in PIE
7	$M_2 - C_6$	No	No	No
8	$M_2 - \{C_3, C_4, C_6\}$	Yes in PIE	Yes in PIE, NIE	Yes in PIE, NIE
9	$M_2 - \{A * M_1\}$	Yes in PIE	No	No
10a	$\{M_1 - C_1\}$ & $\{M_2 - C_3\}$	Yes in NIE	Yes in NIE	Yes in NIE
11a	$\{M_1 - C_4\}$ & $\{M_2 - C_4\}$	Yes in NIE, PIE	Yes in NIE	Yes in NIE, PIE
12a	$\{M_1 - C_5\}$ & $\{M_2 - C_6\}$	No	Yes in NIE	Yes in NIE

Note: Description column represents CSM or mis-specified models, for example, $M_1 - C_1$ indicates, C_1 was omitted from M_1 equation.

5.2 Cross method comparison

Table 6 displays the results of performance in risk difference scale and risk ratio scales when confounders, mediators, and interactions were correctly specified. With respect to coverage probability, Lange method had best 95% confidence interval (CI) coverage compared to the other two approaches.

5.2a Comparison of Lange, Steen and Hong methods in estimating total effects

Because three methods had the same mis-specification in scenarios 1-9, we did method comparison using CSM (CSM1 and CSM2 for Steen method) and scenarios 1-9. The robustness of the three methods in RD and RR scales for TE estimation were shown in Figure 6 and Table 10.

Low values (close to 0) of bias and RMSE indicate low bias. High values of coverage probability (The closer to 95%, the better.) indicate better performance. As shown in Table 10, if the color was red, the method performed the best among three methods based on the criteria we discussed previously; green, the middle; and blue, the worst. The higher the total numbers of red are, the better overall performance for that method.

Lange method had better coverage in RD and RR scales for TE than the other two methods, and Steen had the poorest coverage in RD and RR scales for TE in most scenarios (Figured 6E and 6F, and Table 16). Hong method had the smallest bias and RMSE in RD and RR scales for TE. Given that the bias and RMSE for TE were small for all three methods, we compared the performance of the three methods in TE estimation using coverage.

Lange method had a score of 10 in coverage (Table 10), which indicates that Lange method was more robust in TE estimation comparing to Steen and Hong methods.

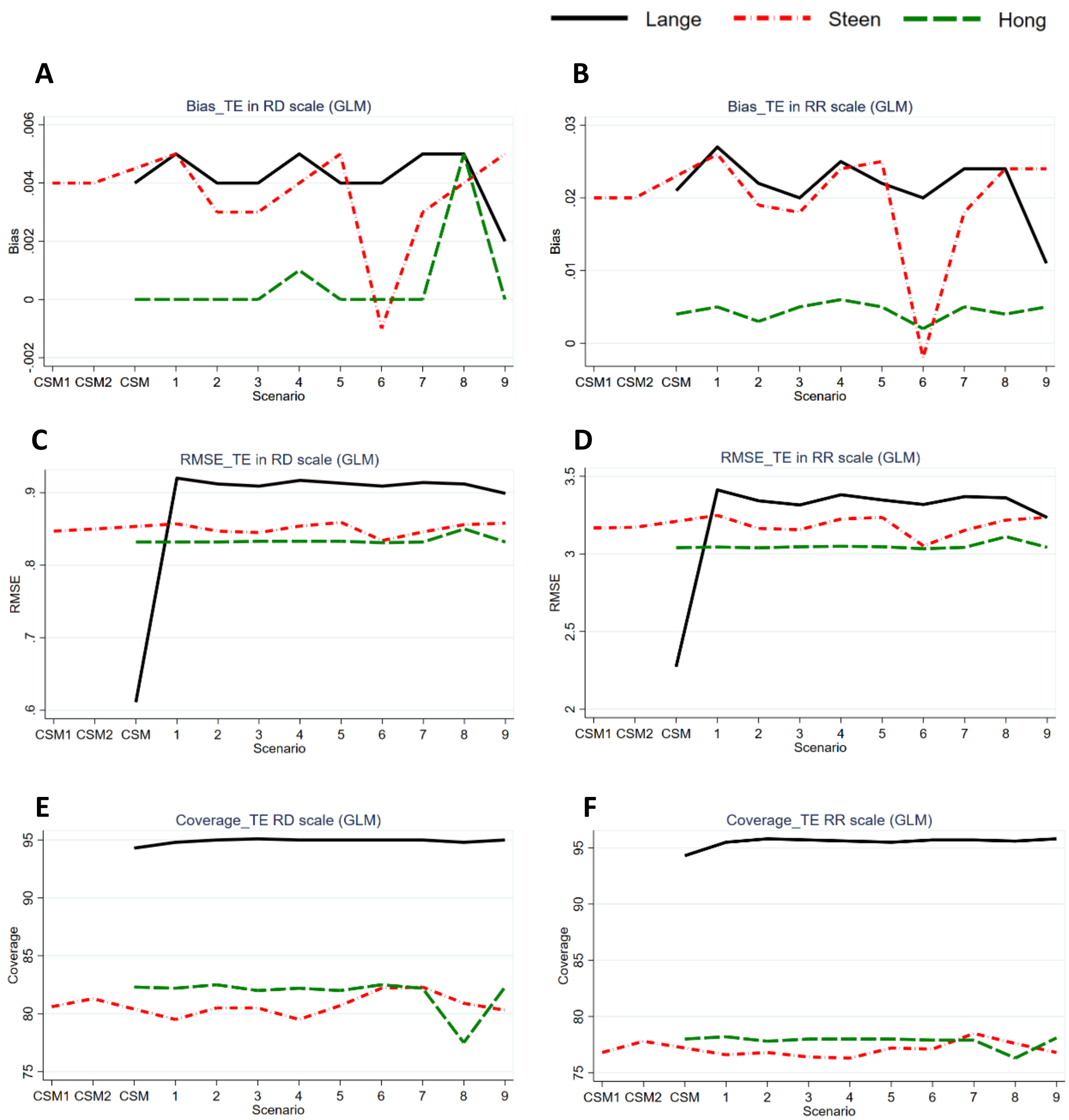

Figure 6. Comparison of Lange method, Steen method and Hong method in TE estimation

Black solid line represents Lange method; red dot-dashed line represents Steen method; green long-dashed line represents Hong method.

Bias in RD scale (A) and in RR scale (B); RMSE in RD scale (C) and RR scale (D); coverage probability in RD scale (E) and RR scale (F).

Table 10 Summarizing comparison of Lange, Steen and Hong methods in TE estimation in RD scale

TE in RD scale	Bias			Coverage			RMSE		
Scenario	Lange	Steen	Hong	Lange	Steen	Hong	Lange	Steen	Hong
CSM	blue ●	NA	red ●	red ▲	NA	green ▲	red ■	NA	green ■
CSM1	NA	green ●	NA	NA	blue ▲	NA	NA	blue ●	NA
CSM2	NA	green ●	NA	NA	blue ▲	NA	NA	blue ●	NA
1	blue ●	green ●	red ●	red ▲	blue ▲	green ▲	blue ■	green ■	red ■
2	blue ●	green ●	red ●	red ▲	blue ▲	green ▲	blue ■	green ■	red ■
3	blue ●	green ●	red ●	red ▲	blue ▲	green ▲	blue ■	green ■	red ■
4	blue ●	green ●	red ●	red ▲	blue ▲	green ▲	blue ■	green ■	red ■
5	blue ●	green ●	red ●	red ▲	blue ▲	green ▲	blue ■	green ■	red ■
6	blue ●	red ●	green ●	red ▲	blue ▲	green ▲	blue ■	green ■	red ■
7	blue ●	green ●	red ●	red ▲	blue ▲	green ▲	blue ■	green ■	red ■
8	green ●	red ●	blue ●	red ▲	green ▲	blue ▲	blue ■	green ■	red ■
9	green ●	blue ●	red ●	red ▲	blue ▲	green ▲	blue ■	green ■	red ■
Total	**0**	**2**	**8**	**10**	**0**	**0**	**1**	**0**	**9**

Note:

Smallest bias red ● Middle bias green ● Largest bias blue ● (The smaller, the better)

Largest coverage red ▲ Middle coverage green ▲ Smallest coverage blue ▲ (The larger, the better)

Smallest RMSE red ■ Middle RMSE green ■ Largest bias blue ■ (The smaller, the better)

Low values (or absolute values) of bias indicate low bias.

High values of coverage probability (The closer to 95%, the better.) indicate better performance.

Low values (or absolute values) of RMSE reflect low bias, high precision, or some combination of the two.

5.2b Comparison of Lange, Steen and Hong methods in estimating natural direct effect

Because the bias and RMSE for NDE were relatively small for all three methods, we compared the performance of the three methods in NDE estimation using coverage (Figure 7). Lange method had a score of 10 in coverage (Table 11), suggesting that Lange method performed better in NDE estimation comparing to Steen and Hong methods.

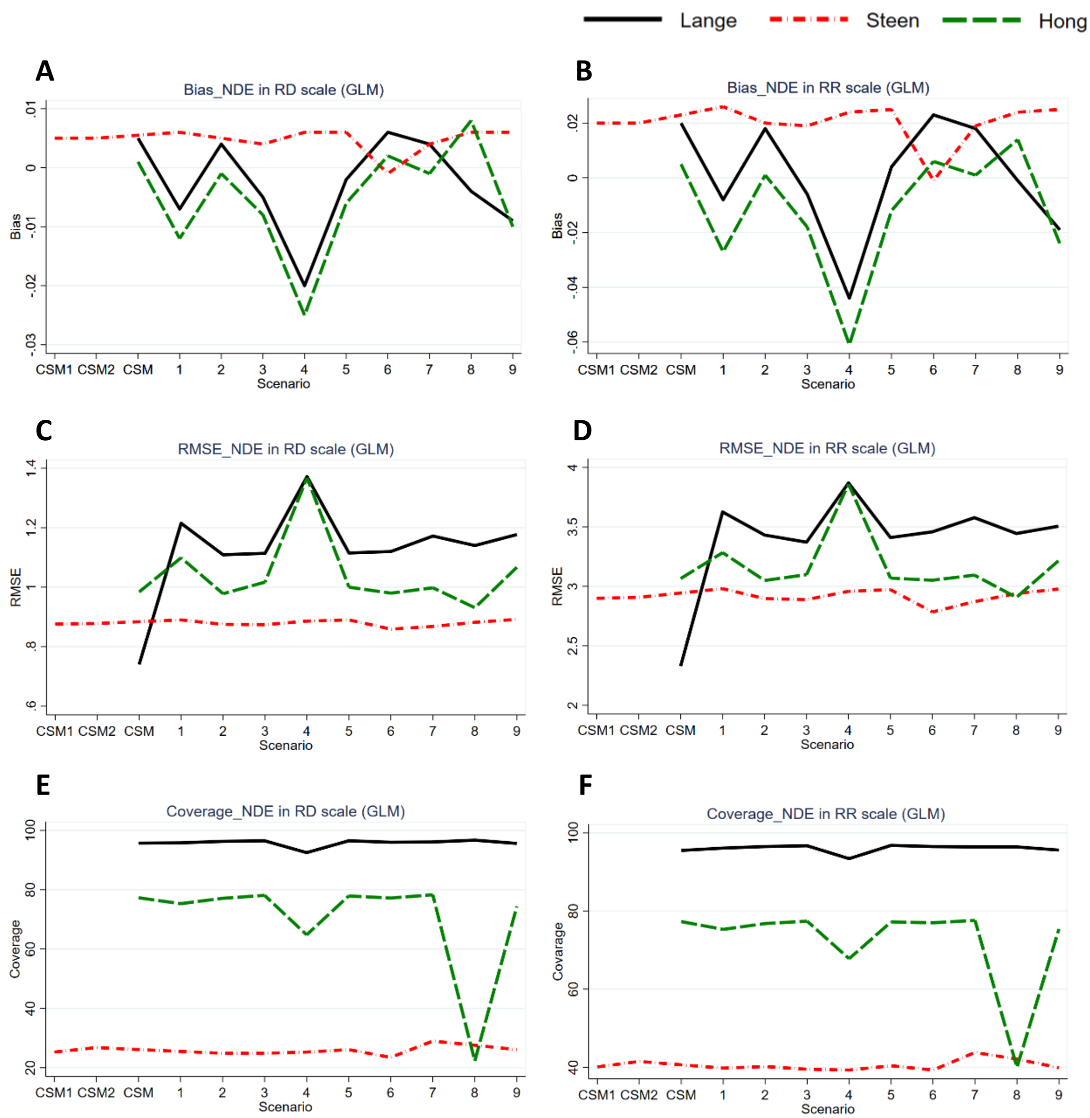

Figure 7. Comparison of Lange method, Steen method and Hong method in NDE estimation

Black solid line represents Lange method; red dot-dashed line represents Steen method; green long-dashed line represents Hong method.

Bias in RD scale (A) and in RR scale (B); RMSE in RD scale (C) and RR scale (D); coverage probability in RD scale (E) and RR scale (F).

Table 11 Summarizing comparison of Lange, Steen and Hong methods in NDE estimation in RD scale

NDE in RD scale	Bias			Coverage			RMSE		
Scenario	Lange	Steen	Hong	Lange	Steen	Hong	Lange	Steen	Hong
CSM	● (blue)	NA	● (red)	▲ (red)	NA	▲ (green)	■ (red)	NA	■ (green)
CSM1	NA	● (green)	NA	NA	▲ (blue)	NA	NA	● (blue)	NA
CSM2	NA	● (green)	NA	NA	▲ (blue)	NA	NA	● (blue)	NA
1	● (red)	● (green)	● (blue)	▲ (red)	▲ (blue)	▲ (green)	■ (blue)	■ (red)	■ (green)
2	● (green)	● (blue)	● (red)	▲ (red)	▲ (blue)	▲ (green)	■ (blue)	■ (red)	■ (green)
3	● (green)	● (red)	● (blue)	▲ (red)	▲ (blue)	▲ (green)	■ (blue)	■ (red)	■ (green)
4	● (green)	● (red)	● (blue)	▲ (red)	▲ (blue)	▲ (green)	■ (blue)	■ (red)	■ (green)
5	● (red)	● (green)	● (blue)	▲ (red)	▲ (blue)	▲ (green)	■ (blue)	■ (red)	■ (green)
6	● (blue)	● (red)	● (green)	▲ (red)	▲ (blue)	▲ (green)	■ (blue)	■ (red)	■ (green)
7	● (blue)	● (green)	● (red)	▲ (red)	▲ (blue)	▲ (green)	■ (blue)	■ (red)	■ (green)
8	● (red)	● (green)	● (blue)	▲ (red)	▲ (green)	▲ (blue)	■ (blue)	■ (red)	■ (green)
9	● (green)	● (red)	● (blue)	▲ (red)	▲ (blue)	▲ (green)	■ (blue)	■ (red)	■ (green)
Total	**3**	**4**	**3**	**10**	**0**	**0**	**1**	**9**	**0**

Note:

Smallest bias ● (red) Middle bias ● (green) Largest bias ● (blue) (The smaller, the better)

Largest coverage ▲ (red) Middle coverage ▲ (green) Smallest coverage ▲ (blue) (The larger, the better)

Smallest RMSE ■ (red) Middle RMSE ■ (green) Largest bias ■ (blue) (The smaller, the better)

Low values (or absolute values) of bias indicate low bias.

High values of coverage probability (The closer to 95%, the better.) indicate better performance.

Low values (or absolute values) of RMSE reflect low bias, high precision, or some combination of the two.

5.2c Comparison of Lange, Steen and Hong methods in estimating natural indirect effect

As shown in Figure 8 and Table 12, Lange method had smaller bias and RMSE for NIE relative to Steen and Hong methods. Moreover, Lange had higher coverage for NIE estimation in 9 out of 10 scenarios (Table 12). Hong method had different rationale in estimating NIE from Lange and Steen methods, and had poorest performance in bias, RMSE and coverage in NIE estimation in almost all scenarios (Table 12).

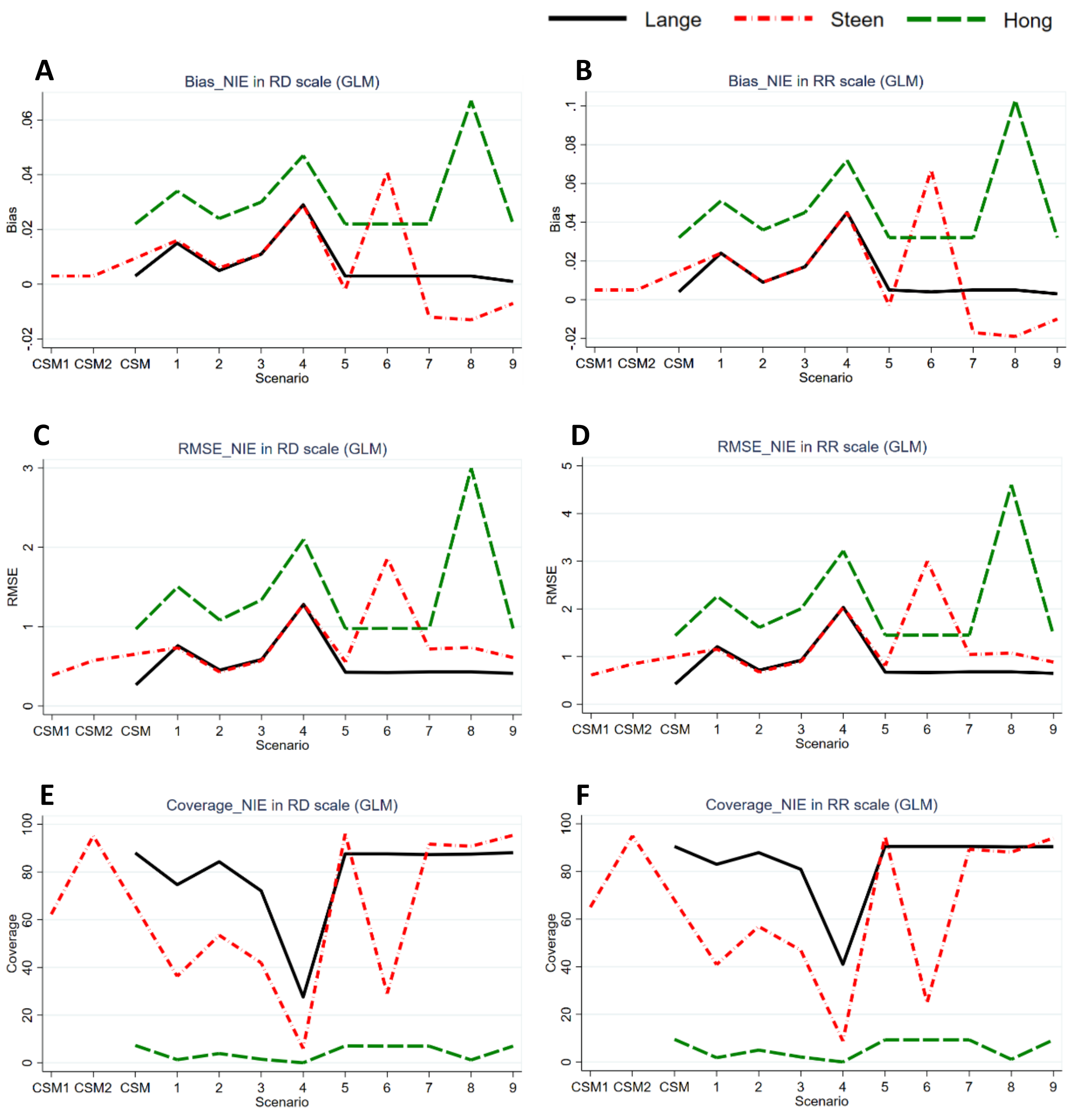

Figure 8. Comparison of Lange method, Steen method and Hong method in NIE estimation

Black solid line represents Lange method; red dot-dashed line represents Steen method; green long-dashed line represents Hong method.

Bias in RD scale (A) and in RR scale (B); RMSE in RD scale (C) and RR scale (D); coverage probability in RD scale (E) and RR scale (F).

Table 12. Summarizing comparison of Lange, Steen and Hong methods in NIE estimation in RD scale

NIE in RD scale	Bias			Coverage			RMSE		
Scenario	Lange	Steen	Hong	Lange	Steen	Hong	Lange	Steen	Hong
CSM	red ●	NA	blue ●	red ▲	NA	blue ▲	red ■	NA	blue ■
CSM1	NA	green ●	NA	NA	green ▲	NA	NA	green ●	NA
CSM2	NA	green ●	NA	NA	green ▲	NA	NA	green ●	NA
1	red ●	green ●	blue ●	red ▲	green ▲	blue ▲	green ■	red ■	blue ■
2	red ●	green ●	blue ●	red ▲	green ▲	blue ▲	green ■	red ■	blue ■
3	red ●	green ●	blue ●	red ▲	green ▲	blue ▲	green ■	red ■	blue ■
4	red ●	green ●	blue ●	red ▲	green ▲	blue ▲	red ■	green ■	blue ■
5	green ●	red ●	blue ●	green ▲	red ▲	blue ▲	red ■	green ■	blue ■
6	red ●	blue ●	green ●	red ▲	green ▲	blue ▲	red ■	blue ■	green ■
7	red ●	green ●	blue ●	green ▲	red ▲	blue ▲	red ■	green ■	blue ■
8	red ●	green ●	blue ●	green ▲	red ▲	blue ▲	red ■	green ■	blue ■
9	red ●	green ●	blue ●	green ▲	red ▲	blue ▲	red ■	green ■	blue ■
Total	**9**	**1**	**0**	**6**	**4**	**0**	**7**	**3**	**0**

Note:

Smallest bias red ● Middle bias green ● Largest bias blue ● (The smaller, the better)

Largest coverage red ▲ Middle coverage green ▲ Smallest coverage blue ▲ (The larger, the better)

Smallest RMSE red ■ Middle RMSE green ■ Largest bias blue ■ (The smaller, the better)

Low values (or absolute values) of bias indicate low bias.

High values of coverage probability (The closer to 95%, the better.) indicate better performance.

Low values (or absolute values) of RMSE reflect low bias, high precision, or some combination of the two.

5.2d Comparison of Lange, Steen and Hong methods in estimating partial indirect effect

As shown in Figure 9 and Table 13, Lange method had smallest bias and RMSE in most scenario among the three methods (Figure 9 and Table 13). Lange method had highest coverage for PIE in all scenarios (Table 13). The performance of Steen method ranked the second. Hong method it performed poorly in PIE estimation in almost all scenarios.

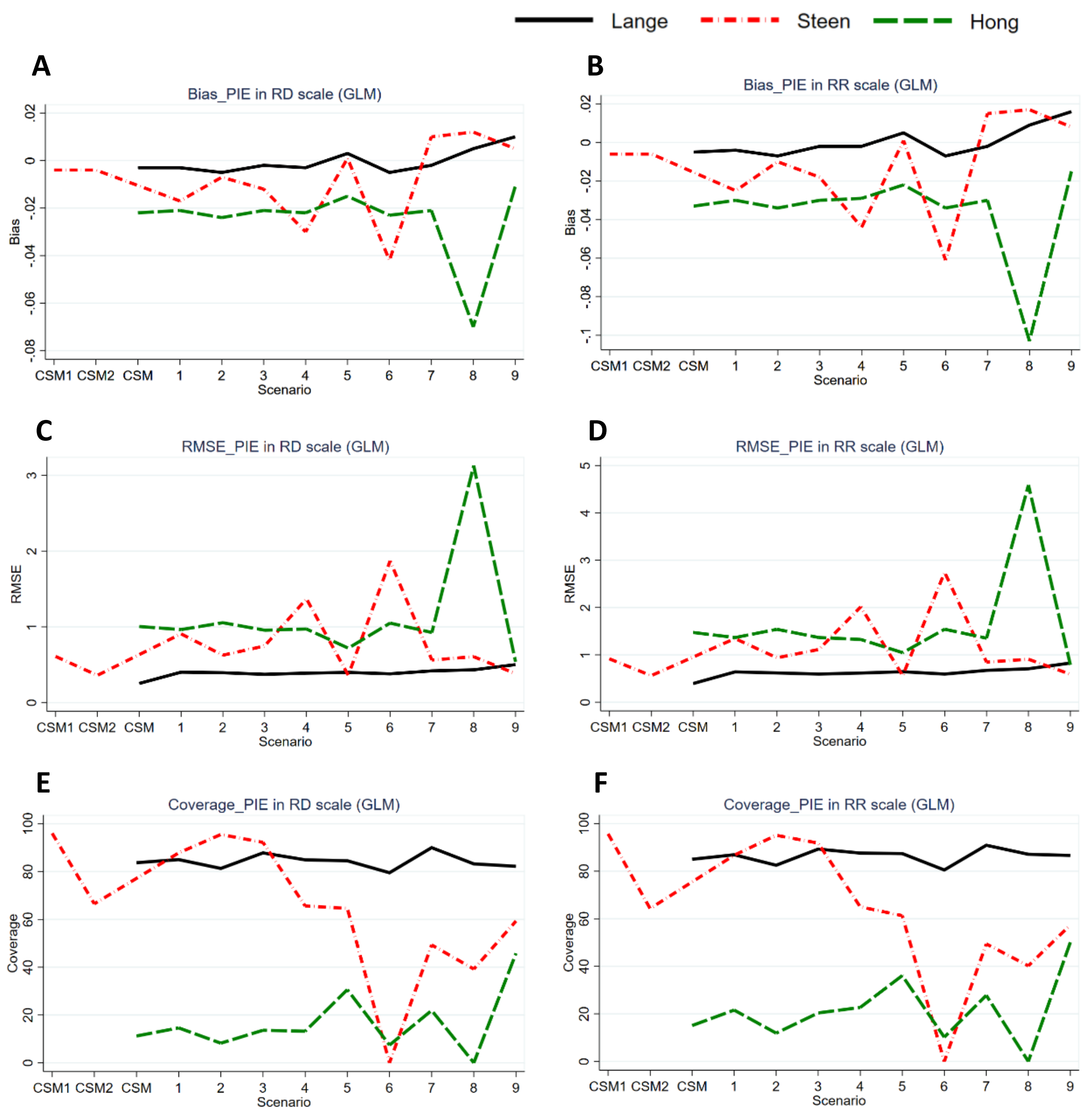

Figure 9. Comparison of Lange method, Steen method and Hong method in PIE estimation

Black solid line represents Lange method; red dot-dashed line represents Steen method; green long-dashed line represents Hong method.

Bias in RD scale (A) and in RR scale (B); RMSE in RD scale (C) and RR scale (D); coverage probability in RD scale (E) and RR scale (F).

Table 13. Summarizing comparison of Lange, Steen and Hong methods in PIE estimation

PIE in RD scale	Bias			Coverage			RMSE		
Scenario	Lange	Steen	Hong	Lange	Steen	Hong	Lange	Steen	Hong
CSM	red ●	NA	blue ●	red ▲	NA	blue ▲	red ■	NA	blue ■
CSM1	NA	green ●	NA	NA	green ▲	NA	NA	green ●	NA
CSM2	NA	green ●	NA	NA	green ▲	NA	NA	green ●	NA
1	red ●	green ●	blue ●	red ▲	green ▲	blue ▲	red ■	green ■	blue ■
2	red ●	green ●	blue ●	red ▲	green ▲	blue ▲	red ■	green ■	blue ■
3	red ●	green ●	blue ●	red ▲	green ▲	blue ▲	red ■	green ■	blue ■
4	red ●	green ●	blue ●	red ▲	green ▲	blue ▲	red ■	blue ■	green ■
5	red ●	green ●	blue ●	red ▲	green ▲	blue ▲	green ■	red ■	blue ■
6	red ●	blue ●	green ●	red ▲	blue ▲	green ▲	red ■	blue ■	green ■
7	red ●	green ●	blue ●	red ▲	green ▲	blue ▲	red ■	green ■	blue ■
8	red ●	green ●	blue ●	red ▲	green ▲	blue ▲	red ■	green ■	blue ■
9	red ●	green ●	blue ●	red ▲	green ▲	blue ▲	green ■	red ■	blue ■
Total	**10**	**0**	**0**	**10**	**0**	**0**	**8**	**2**	**0**

Note:

Smallest bias red ● Middle bias green ● Largest bias blue ● (The smaller, the better)

Largest coverage red ▲ Middle coverage green ▲ Smallest coverage blue ▲ (The larger, the better)

Smallest RMSE red ■ Middle RMSE green ■ Largest bias blue ■ (The smaller, the better)

Low values (or absolute values) of bias indicate low bias.

Low values (or absolute values) of bias indicate low bias.

High values of coverage probability (The closer to 95%, the better.) indicate better performance.

Low values (or absolute values) of RMSE reflect low bias, high precision, or some combination of the two.

CHAPTER 6. CONCLUSION

To understand the mechanisms with two sequential mediators, it is necessary to investigate the counterfactual framework (3, 10, 16). In this thesis, motivated by previous research (9), we considered two-sequential-mediator models with binary mediators, a binary outcome and a set of binary confounders. Before this study, Lange et al (8) had performed the only simulation that we knew of that allowed different sets of confounders for different $\mathrm{A}-\mathrm{M}-\mathrm{Y}$ relationships; but they considered only one mediator and three confounders. We extended the scenarios with mis-specifications by omitting one or multiple confounders, or deleting an interaction.

By comparing their robustness in bias, RMSE and coverage in various scenarios, we concluded that Lange method was more resilient to mis-specifications and performed better in NIE and PIE estimation in most scenarios compared to Steen and Hong methods (Figures 8 and 9, Tables 12 and 13). Hong method had largest bias and RMSE, and lowest coverage for NIE and PIE among the three methods. In addition, Lange method showed the same changes in bias, RMSE, and coverage as we expected in most scenarios when mis-specification occurred.

Coverage for all causal effects estimated using Steen and Hong methods were much smaller than the expected 95% in most scenarios (Figures 4 and 5). Low coverage was likely due to the fact that we did not use bootstrapping to obtain coverage, but employed default standard errors for parameter estimates (section 4.2). Bootstrapping will likely provide better coverage as the calculation of the standard errors and confidence interval is more accurate (3).

APPENDICES

APPENDIX A: Validity of the Modified Lange Method

Consistency: $Y^{a,m_1,m_2} = Y$ when $A = a; M_1^1 = M_1; M_2^1 = M_2$

Positivity: $0 < \Pr(A = a|\, C = c) < 1;\ 0 < \Pr(M_k = m_k|\, A = a, C = c) < 1$

No unmeasured confounders: $Y^{a,m_1,m_2} \perp A \mid C;\ \ M_i^a \perp A \mid C;\ \ Y^{a,m_1,m_2} \perp M^i \,\middle|\, A = c, C$

$i = 1,2$

Identification assumptions (Extended sequential ignorability):

$Y^{a',m_1,m_2} \perp M_i^a \,\middle|\, C$ and $M_i^a \perp M_j^{a'} \,\middle|\, C$ for all a, a', i, j where $i \neq j, i = 1,2, j = 1,2$

Under above-mentioned assumptions, we can estimate $E\left(Y^{a,M_1^{a_1},M_2^{a_2}}\right)$ in the mediation analysis with two consecutive mediators (M_1 and M_2).

$$E\left(Y^{a,M_1^{a_1},M_2^{a_2}}\right) = \sum_{m_1,m_2} \sum_{c} E\big(Y^{a,m_1,m_2} | M_1^{a_1} = m_1, M_2^{a_2} = m_2, C = c\big)$$

$$* \Pr(M_1^{a_1} = m_1, M_2^{a_2} = m_2, |\, C = c) * \Pr(C = c)$$

$$= \sum_{m_1,m_2} \sum_{c} E(Y^{a,m_1,m_2} | C = c) * \Pr(M_1^{a_1} = m_1|\, C = c) * \Pr(M_2^{a_2} = m_1|\, C = c) * \Pr(C = c)$$

$$= \sum_{m_1,m_2} \sum_{c} E(Y^{a,m_1,m_2} | M_1 = m_1, M_2 = m_2, A = a, C = c) * \Pr\big(M_1^{a_1} = m_1 \big| A = a_1, C = c\big)$$

$$* \Pr\big(M_2^{a_2} = m_1 \big|\, A = a_2, C = c) * \Pr(C = c)$$

$$= \sum_{m_1,m_2} \sum_{c} E(Y | M_1 = m_1, M_2 = m_2, A = a, C = c) * \Pr(M_1 = m_1 | A = a_1, C = c)$$

$$* \Pr(M_2 = m_1 |\, A = a_2, C = c) * \Pr(C = c)$$

$$= \sum_{y} \sum_{m_1,m_2} \sum_{c} y * \Pr(Y = y | M_1 = m_1, M_2 = m_2, A = a, C = c)$$

$$* \Pr(M_1 = m_1 | A = a_1, C = c) * \Pr(M_2 = m_1 |\, A = a_2, C = c) * \Pr(C = c)$$

$$= \sum_{a} \sum_{y} \sum_{m_1,m_2} \sum_{c} y * I(A = a) * \Pr(Y = y | M_1 = m_1, M_2 = m_2, A = a, C = c)$$

$$* \Pr(M_1 = m_1 | A = a, C = c) * \frac{\Pr(M_1 = m_1 | A = a_1, C = c)}{\Pr(M_1 = m_1 | A = a, C = c)}$$

$$* \Pr(M_2 = m_2 | A = a, C = c) * \frac{\Pr(M_2 = m_2 | A = a_2, C = c)}{\Pr(M_2 = m_2 | A = a, C = c)}$$

$$* \frac{\Pr(C = c)}{\Pr(A = a | C = c)}$$

$$= \sum_{a} \sum_{y} \sum_{m_1, m_2} \sum_{c} y * I(A = a) * \Pr(Y = y | M_1 = m_1, M_2 = m_2, A = a, C = c)$$

$$* \Pr(M_1 = m_1 | A = a, C = c) * \frac{\Pr(M_1 = m_1 | A = a_1, C = c)}{\Pr(M_1 = m_1 | A = a, C = c)}$$

$$* \Pr(M_2 = m_2 | A = a, C = c) * \frac{\Pr(M_2 = m_2 | A = a_2, C = c)}{\Pr(M_2 = m_2 | A = a, C = c)}$$

$$* \frac{1}{\Pr(A = a)}$$

$$= \sum_{a} \sum_{y} \sum_{m_1, m_2} \sum_{c} y * I(A = a) * \Pr(Y = y, M_1 = m_1, M_2 = m_2, A = a, C = c) * W$$

$= E(Y * I(A = a) * W)$ (Where W is the weight.)

APPENDIX B: Proof of RMPW for estimating counterfactual outcomes of consecutive mediators

To obtain unbiased estimates of the causal effects applying RMPW method, stronger assumptions about the sequential ignorability are required as follows:

(i)

$$A \perp Y^{a,m_1,m_2} \,\big|\, C_1, C_2, C_3, C_4, C_5, C_6$$

$$A \perp M_1, M_2 \,\big|\, C_1, C_2, C_3, C_4, C_5, C_6$$

Exposure *A* is independent of all the potential outcomes and the potential mediators given the observed pretreatment covariates C.

(ii)

$$M_1 \perp Y^{a,m_1,m_2} \,\big|\, A, C_1, C_2, C_3, C_4, C_5, C_6$$

Given *A* and **C**, the assignment of M_1 is independent of all the potential outcomes and the potential mediators.

(iii*)

$$M_2 \perp Y^{a,m_1,m_2} \,\big|\, A, M_1, C_1, C_2, C_3, C_4, C_5, C_6$$

Given A, M_1, and C, the assignment of M_2 is independent of all the potential outcomes.

Individuals are assigned at random to $A = 1$ or $A = 0$ at the first stage of the experiment; those in the same treatment group a are assigned at random to different values of the first mediator M_1^a at the second stage for $a = 0, 1$; those in the same treatment group a and with the same mediator value m_1 are assigned at random to different values of the second mediator M_2^{a,m_1} at the third stage for a = 0,1 and for all possible values of m_1.

Note: $M_2^{0,m_1^0} = M_2^0$

$$\mathbf{E}\left(\mathbf{Y}^{\mathbf{1},\mathbf{M_1^0},\mathbf{M_2^{0,M_1^0}}}\right) \equiv E\left\{E\left(Y^{1,M_1^0,M_2^{0,M_1^0}} | C\right)\right\} = E\left\{E\left(Y^{1,M_1^0,M_2^{0,M_1^0}} | A = 1, C\right)\right\}$$

$$= \int_{x,m_1,m_2,y}\int\int\int y * f(Y^{1,m_1,m_2} = y|A = 1, M_1^0 = m_1, M_2^{0,m_1^0} = m_2, C = c)$$

$$* \left(\Pr\left(M_2^{0,m_1^0} = m_2\middle| A = 1, M_1^0 = m_1, C = c\right) * \Pr(M_1^0 = m_1\middle|A = 1, C = c)\right.$$

$$* h(C = c)\, dydm_2\, dm_1 dx$$

$$= \int_{x,m_1,m_2,y}\int\int\int y$$

$$* \left\{ f(Y^{1,m_1,m_2} = y, M_2^0 = m_2|A = 1, M_1^0 = m_1, C = c) \middle/ \Pr(M_2^0 = m_2|A = 1, M_1^0 = m_1, C = c) \right\}$$

$$* \Pr(M_2^0 = m_2|A = 1, M_1^0 = m_1, C = c)) * \Pr(M_1^0 = m_1\middle|A = 1, C = c)$$

$$* H(C = c)\, dydm_2\, dm_1 dx$$

$$= \int_{x,m_1,m_2,y}\int\int\int y$$

$$* \left\{ f(Y^{1,m_1,m_2} = y, M_2^0 = m_2|A = 1, M_1^1 = m_1, C = c) \middle/ \Pr(M_2^0 = m_2|A = 1, M_1^1 = m_1, C = c) \right\}$$

$$* \Pr(M_2^0 = m_2|A = 1, M_1^0 = m_1, C = c)) * \Pr(M_1^0 = m_1\middle|A = 0, C = c)$$

$$* H(C = c)\, dydm_2\, dm_1 dx$$

$$= \int_{x,m_1,m_2,y}\int\int\int y * (Y^{1,m_1,m_2} = y|A = 1, M_1^1 = m_1, M_2^0 = m_2, C = c)$$

$$* \Pr(M_2^0 = m_2|A = 1, M_1^0 = m_1, C = c)) * \Pr(M_1^0 = m_1\middle|A = 0, C = c)$$

$$* H(C = c)\, dydm_2\, dm_1 dx$$

$$= \int_{x,m_1,m_2,y}\int\int\int y * (Y^{1,m_1,m_2} = y|A = 1, M_1^1 = m_1, M_2^1 = m_2, C = c)$$

$$* \Pr(M_2^1 = m_2|A = 1, M_1^1 = m_1, C = c)) * \Pr(M_1^1 = m_1\middle|A = 1, C = c)$$

$$* \frac{\Pr(M_1^0 = m_1|A = 0, C = c) * \Pr(M_2^0 = m_2|A = 0, M_1^0 = m_1, C = c)}{\Pr(M_1^1 = m_1|A = 1, C = c) * \Pr(M_2^1 = m_2|A = 1, M_1^1 = m_1, C = c)}$$

$$* h(C = c)\, dydm_2\, dm_1 dx$$

$$= E(Y^*|A = 1) \text{ where } Y^* = WY$$

RMPW weight

$$W = \frac{\Pr(M_1^0 = m_1|A = 0, C = c) * \Pr(M_2^0 = m_2|A = 0, M_1^0 = m_1, C = c)}{\Pr(M_1^1 = m_1|A = 1, C = c) * \Pr(M_2^1 = m_2|A = 1, M_1^1 = m_1, C = c)}$$

$$= \frac{\theta_{M_1^0 = m_1}}{\theta_{M_1^1 = m_1}} * \frac{\theta_{M_2^{0,m_1} = m_2}}{\theta_{M_2^{1,m_1} = m_2}}$$

Note: $M_2^{0,m_1^0} = M_2^0$

$$\mathbf{E}\left(\mathbf{Y}^{\mathbf{1},\mathbf{M}_1^1,\mathbf{M}_2^{\mathbf{0},\mathbf{M}_1^0}}\right) \equiv E\left\{E\left(Y^{1,M_1^1,M_2^{0,M_1^0}} |C\right)\right\} = E\left\{E\left(Y^{1,M_1^1,M_2^{0,M_1^0}} |A = 1, C\right)\right\}$$

$$= \int\int\int\int_{x, m_1, m_2, y} y * f(Y^{1,m_1,m_2} = y|A = 1, M_1^1 = m_1, M_2^0 = m_2, C = c)$$

$$* \left(\Pr(M_2^0 = m_2 \middle| A = 1, M_1^1 = m_1, C = c\right) * \Pr(M_1^1 = m_1 \middle| A = 1, C = c)$$

$$* h(C = c)\, dy dm_2\, dm_1 dx$$

$$= \int\int\int\int_{x, m_1, m_2, y} y * \ f(Y^{1,m_1,m_2} = y|A = 1, M_1^1 = m_1, M_2^1 = m_2, C = c)$$

$$* \left\{\Pr(M_2^0 = m_2, M_1^1 = m_1|A = 0, C = c)) \middle/ \Pr(M_2^1 = m_2, M_1^1 = m_1|A = 0, C = c))\right\}$$

$$* \Pr(M_1^1 = m_1|A = 1, C = c) * h(C = c)\, dy dm_2\, dm_1 dx$$

$$= \int\int\int\int_{x, m_1, m_2, y} y * \ f(Y^{1,m_1,m_2} = y|A = 1, M_1^1 = m_1, M_2^1 = m_2, C = c)$$

$$* \left\{\Pr(M_2^0 = m_2, M_1^1 = m_1|A = 1, C = c)) \middle/ \Pr(M_2^1 = m_2, M_1^1 = m_1|A = 1, C = c))\right\}$$

$$* \Pr(M_1^1 = m_1|A = 1, C = c) * h(C = c)\, dy dm_2\, dm_1 dx$$

$$= \int\int\int\int y * \ f(Y^{1,m_1,m_2} = y|A = 1, M_1^1 = m_1, M_2^1 = m_2, C = c)$$

$$* \Pr(M_2^1 = m_2|A = 1, M_1^1 = m_1, C = c) * \Pr(M_1^1 = m_1|A = 1, C = c)$$

$$* \frac{\Pr(M_2^0 = m_2|A = 0, M_1^0 = m_1, C = c)}{\Pr(M_2^1 = m_2|A = 1, M_1^1 = m_1, C = c)} * h(C = c)\, dy dm_2\, dm_1 dx$$

$$= E(Y^*|A = 1) \text{ where } Y^* = WY$$

RMPW weight

$$W = \frac{\Pr(M_2^0 = m_2|A = 0, M_1^0 = m_1, C = c)}{\Pr(M_2^1 = m_2|A = 1, M_1^1 = m_1, C = c)} = \frac{\theta_{M_2^{0,m_1}=m_2}}{\theta_{M_2^{1,m_1}=m_2}}$$

$$\mathbf{E}\left(\mathbf{Y}^{\mathbf{1},\mathbf{M_1^0},\mathbf{M_2}^{\mathbf{1},\mathbf{M_1^1}}}\right) \equiv E\left\{E\left(Y^{1,M_1^0,M_2^{1,M_1^1}}|C\right)\right\} = E\left\{E\left(Y^{1,M_1^0,M_2^{1,M_1^1}}|A = 1, C\right)\right\}$$

$$= \int\int\int\int_{x,m_1,m_2,y} y * f(Y^{1,m_1,m_2} = y|A = 1, M_1^0 = m_1, M_2^1 = m_2, C = c)$$
$$* \left(\Pr(M_2^1 = m_2\middle| A = 1, M_1^0 = m_1, C = c\right) * \Pr(M_1^0 = m_1\middle|A = 1, C = c)$$
$$* h(C = c)\, dydm_2\, dm_1dx$$

$$= \int\int\int\int_{x,m_1,m_2,y} y$$
$$* \left\{ f(Y^{1,m_1,m_2} = y, M_2^1 = m_2|A = 1, M_1^0 = m_1, C = c) \middle/ \Pr(M_2^1 = m_2|A = 1, M_1^0 = m_1, C = c) \right\}$$
$$* \Pr(M_2^1 = m_2|A = 1, M_1^0 = m_1, C = c)) * \Pr(M_1^0 = m_1\middle|A = 1, C = c)$$
$$* h(C = c)\, dydm_2\, dm_1dx$$

$$= \int\int\int\int_{x,m_1,m_2,y} y$$
$$* \left\{ f(Y^{1,m_1,m_2} = y, M_2^1 = m_2|A = 1, M_1^1 = m_1, C = c) \middle/ \Pr(M_2^1 = m_2|A = 1, M_1^1 = m_1, C = c) \right\}$$
$$* \Pr(M_2^1 = m_2|A = 1, M_1^1 = m_1, C = c)) * \Pr(M_1^0 = m_1\middle|A = 0, C = c)$$
$$* h(C = c)\, dydm_2\, dm_1dx$$

$$= \int\int\int\int_{x,m_1,m_2,y} y * (Y^{1,m_1,m_2} = y|A = 1, M_1^1 = m_1, M_2^1 = m_2, C = c)$$
$$* \Pr(M_2^1 = m_2|A = 1, M_1^1 = m_1, C = c)) * \Pr(M_1^0 = m_1\middle|A = 0, C = c)$$
$$* h(C = c)\, dydm_2\, dm_1dx$$

$$= \int\int\int\int_{x,m_1,m_2,y} y * (Y^{1,m_1,m_2} = y|A = 1, M_1^1 = m_1, M_2^1 = m_2, C = c)$$
$$* \Pr(M_2^1 = m_2|A = 1, M_1^1 = m_1, C = c)) * \Pr(M_1^1 = m_1\middle|A = 1, C = c)$$

$$* \frac{\Pr(M_1^0 = m_1|A = 0, C = c)}{\Pr(M_1^1 = m_1|A = 1, C = c)} * h(C = c)\, dydm_2\, dm_1dx$$

$= E(Y^*|A = 1)$ where $Y^* = WY$

RMPW weight

$$W = \frac{\Pr(M_1^0 = m_1 | A = 0, C = c)}{\Pr(M_1^1 = m_1 | A = 1, C = c)} = \frac{\theta_{M_1^0 = m_1}}{\theta_{M_1^1 = m_1}}$$

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