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Abstract

The main aim of mediation analysis is to study the direct and indirect effects of an exposure on an outcome. To date, the literature on mediation analysis with multiple mediators has mainly focused on continuous and dichotomous outcomes. However, the development of methods for multiple mediation analysis of survival outcome is still limited. Here we extend to survival outcomes a method for multiple mediation analysis based on the computation of appropriate weights. The approach considered has the advantage of not requiring specific models for mediators, allowing non-independent mediators of any nature and not relying on the assumption of rare outcomes. Simulation studies show a good performance of the proposed estimator, in terms of bias and coverage probability. The method is further applied to an example from a published study on mortality for prostate cancer aimed at understanding to what extent the effect of DNA methyltransferase genotype on mortality was explained by DNA methylation and tumor aggressiveness. The approach can be used to quantify the marginal time-dependent direct and indirect effects carried by multiple indirect pathways and a code is provided to facilitate its application.

Keywords: multiple mediation analysis, proportional hazards model, pure direct effect, weighting approach, total indirect effect

Abbreviations: pure direct effect (PDE), total indirect effect (TIE), total effect (TE), DNA methyltransferase 3b (DNMT3b), CI (confidence interval)

In medical and epidemiological research it is often of interest to understand the biological or mechanistic pathways that contribute to the effect of an exposure on an outcome. The aim of mediation analysis is to disentangle the total effect into the indirect effect, i.e. the effect exerted by intermediate variables (mediators), and the direct effect, i.e. the effect involving pathways independent of the hypothesized mediators.

A first approach to mediation analysis was proposed by Baron and Kenny in 1986 (1). The theory was later on generalized through a counterfactual approach that provided broader definitions of the direct and indirect effects allowing the presence of nonlinearities and interactions between the exposure and the mediators in the models for the outcome (2-6).

In the counterfactual framework, methods to estimate the direct and indirect effects differ according to the type of outcome. As far as the survival framework is concerned, a mediation approach involving a single mediator was firstly proposed in 2011 by Lange et al. (7), where an additive hazard model was employed to model the time to an event as the outcome of interest. Consequently Vanderweele (8) discussed several effect measures in survival analysis and extended Lange's approach using both an accelerated failure time model and the Cox proportional hazards model with a rare outcome. These standard approaches in the presence of a single mediator were based on combining parameter estimates from the models for the outcome and for the mediator respectively, but the former required a normal continuous mediator and the latter rare outcomes. Tchetgen Tchetgen (9) derived new estimators for mediation analysis for proportional hazards and additive hazards models with appealing robustness properties. Lange (10) developed a weighting approach for the proportional hazards model with a non-rare outcome. In a more recent work, Wang and Albert proposed a mediation formula approach for survival outcome with a normally distributed mediator (11).

Several methods have also been introduced to study mediation effects for scenarios where multiple mediators are considered (12-21), but the focus was on survival analysis only for some of them (12, 14, 15, 17-20). The purpose of the present paper is to show the extension to survival outcome of the weighting approach for multiple mediators proposed by Vanderweele et al. (13) focusing on proportional hazards models. We chose to extend this method because of the many advantages that characterize its employment. It is easily implementable in the presence of multiple mediators not necessarily independent, it does not require specific models for the mediators thus avoiding the problem of model incompatibility and, similarly to the other weighting approaches, it does not rely on the assumption of rare outcomes.

The paper is organized as follows. First we provide definitions and assumptions, then we describe how the approach can be implemented in practice. We show the results obtained from simulation outcomes and a real application on a study on mortality for prostate cancer patients. Finally we discuss the proposed methodology.

Definitions and assumptions

Let the non-negative random variable T denote the time until the occurrence of the event of interest and let U denote the censoring time. Hence (Y, Δ) are the observed data, where $Y = \min(T,U)$, $\Delta = I(T \leq U)$ and $I(\cdot)$ is the indicator function. Let $S_T(t)$ be the survival function, $\lambda_T(t)$ be the hazard function and $f_T(t)$ be the density function at time t. Let A be a dichotomous or a categorical exposure, with a and a^* two possible values of A, $\boldsymbol{M} = (M^1, \cdots, M^k)$ be the vector of multiple mediators and $\boldsymbol{C}$ be the vector of the baseline measured confounders that may affect $A - \boldsymbol{M}$, $\boldsymbol{M} - T$ and $A - T$ associations**.** We explicitly state that the measurements of A, $\boldsymbol{M}$ and C respects their causal ordering and precedes possible censoring. By assuming the independence between T and U conditional on A, $\boldsymbol{M}$, and C, the

functions $S_T(t)$, $\lambda_T(t)$ and $f_T(t)$ can be identified and consistently estimated using the observed data. For a formal definition of this assumption see Web Appendix 1.

Suppose to be interested in evaluating how much of the effect of *A* on *T* is mediated through ***M*** jointly and through pathways other than through ***M***. Within the context of mediation in survival analysis, the decomposition of the total effect of an exposure on the outcome in the indirect and direct effects can be expressed in different ways and scales (8). We will consider here the decomposition on multiplicative scale in terms of hazard functions. By indicating with $\lambda_{T^a}(t)$ the hazard corresponding to a potential survival time had the exposure *A* been set at *a* and with $\lambda_{T^{a,M^{a^*}}}(t)$ the hazard corresponding to a potential survival time under the indicated manipulation of *A* and ***M*** (specifically exposure *A* was set to *a*, but the mediators ***M*** were set to their potential values if *A* had been set to a^*), we can give the following formal definitions in terms of hazard functions:

- total effect (TE), $TE(t) = \lambda_{T^a}(t)/\lambda_{T^{a^*}}(t)$;

- pure direct effect (PDE), $PDE(t) = \lambda_{T^{a,M^{a^*}}}(t)/\lambda_{T^{a^*,M^{a^*}}}(t)$;

- total indirect effect (TIE), $TIE(t) = \lambda_{T^{a,M^a}}(t)/\lambda_{T^{a,M^{a^*}}}(t)$.

Briefly, the $TE(t)$ expresses how much the hazard at time *t* would change if the exposure were changed from level a^* to level a uniformly in the population. The $PDE(t)$ expresses how much the hazard at time *t* would change if the exposure were set at $A = a$ versus $A = a^*$ but the mediators were kept at the level they would have taken had the exposure been set at $A = a^*$. Thus the PDE captures which part of the effect of the exposure on the outcome would be maintained if we were to disable the pathways from the exposure to the mediators. Finally, the $TIE(t)$ expresses how much the hazard at time *t* would change if the exposure were fixed at the level $A = a$ but the mediators were changed from the level they would have taken if $A = a^*$ to the level

they would have taken if $A = a$. Thus the TIE captures the effect of the exposure on the outcome that operates through the mediators. Under the composition assumption $T^a = T^{a,M^a}$ (that is, the value of *T* that would occur if *A* were set to *a* is equal to the value of *T* that would occur if *A* were set to *a* and ***M*** were set to what it would have been if *A* were set to *a;* i.e. under an hypothetical intervention on *a*, interventions on ***M*** to set it to its naturally occurring level $\boldsymbol{M}^a$ have no further effect on the outcome, the total effect is given by the product of total indirect and pure direct effects ($TE(t) = TIE(t) \cdot PDE(t)$).

In order to identify and estimate the causal direct and indirect effects, several assumptions need to be satisfied, specifically the consistency and positivity assumptions, the absence of unmeasured confounders for the exposure-outcome relationship, exposure-mediators relationships, mediators-outcome relationships and the absence of measured/unmeasured mediators-outcome confounders affected by the exposure. However, in some sense, we can now handle violations of this last assumption because if there were such a confounder we could include the variable in the mediator vector ***M*** and this fourth assumption would not be violated. For a formal definition of these assumptions see Web Appendix 1.

The approach we propose in this paper is an extension of the method proposed for continuous and binary outcomes by Vanderweele and Vanstenlandt (13) to survival outcome. The marginal hazard function can be estimated as the ratio between the marginal density and survival functions, both obtained by means of the mediation formula as follows:

$$\lambda_{T^{a,M^{a^*}}}(t) = \frac{E\left[\frac{P(A=a^*)}{P(A=a^*|C)} f_T(t|a,\boldsymbol{M},C)\middle|\ A=a^*\right]}{E\left[\frac{P(A=a^*)}{P(A=a^*|C)} S_T(t|a,\boldsymbol{M},C)\middle|\ A=a^*\right]}$$

A proof of this equation is provided in Web Appendix 1. The approach is then based on inverse probability weighting. Its main feature is that it does not require models for the mediators but only

for the exposure conditional on confounders and for the outcome conditional on the exposure, the mediators and the confounders. The correct specification of these models is a requisite for the validity of the proposed method. Exposure-mediator interactions and interactions between mediators can also be included and the independence between mediators is not necessary. However it allows to consider only binary or categorical exposures.

Specifically we applied the method by using a proportional hazards model for the outcome conditional on the exposure, the mediators and the confounders. Because of non-collapsibility of hazard ratio in the presence of non-rare outcomes, the marginal hazard function could not satisfy the proportionality assumption (22) and hence the pure direct and the total indirect effects may vary over time.

The estimation procedure

The algorithm for the estimation of causal effects requires the computation at any fixed time $\tilde{t}$ of three weighted averages that we will call $Q_1(\tilde{t})$, $Q_2(\tilde{t})$ and $Q_3(\tilde{t})$. If we suppose that $a = 1$ and $a^* = 0$, these weighted averages correspond to the estimates of the counterfactual $\lambda_{T^{1,M^0}}(\tilde{t})$, $\lambda_{T^{0,M^0}}(\tilde{t})$ and $\lambda_{T^{1,M^1}}(\tilde{t})$ respectively. We denote as $\hat{p}_k$ and $\hat{p}_{k|C_i}$ (for $k = 0,1$) the estimates of $P(A = k)$ and $P(A = k|C_i)$ respectively where C_i denotes the actual confounder values for subject *i* and as $\hat{f}_T$ and $\hat{S}_T$ the estimates of f_T and S_T respectively. Furthermore, we indicate by H_0 and H_1 the subsamples of subjects with $A = 0$ and $A = 1$ respectively and by n_0 and n_1 their sizes. The algorithm for the estimation of the effects at a specific time $\tilde{t}$ proceeds as follows:

1. Estimation of $\lambda_{T^{1,M^0}}(\tilde{t})$:

$$Q_1(\tilde{t}) = \frac{\frac{1}{n_0}\sum_{i \in H_0} \frac{\hat{p}_0}{\hat{p}_{0|C_i}} \hat{f}_T(\tilde{t}|A = 1, \boldsymbol{M}_i, C_i)}{\frac{1}{n_0}\sum_{i \in H_0} \frac{\hat{p}_0}{\hat{p}_{0|C_i}} \hat{S}_T(\tilde{t}|A = 1, \boldsymbol{M}_i, C_i)}$$

For each subject with $A = 0$ the hazard function is modelled to obtain a predicted estimate of the density and of the survival functions at time $\tilde{t}$ separately if the subject had had $A = 1$ rather than $A = 0$, but using the individual's own values of mediators and confounders.

Two weighted averages of these predicted values are computed for subjects with $A = 0$ (each subject *i* is given a weight $\frac{\hat{p}_0}{\hat{p}_{0|C_i}}$). Then the ratio of the two weighted averages is computed.

2. Estimation of $\lambda_{T^0,M^0}(\tilde{t})$:

$$Q_2(\tilde{t}) = \frac{\frac{1}{n_0}\sum_{i \in H_0} \frac{\hat{p}_0}{\hat{p}_{0|C_i}} \hat{f}_T(\tilde{t}|A = 0, \boldsymbol{M}_i, C_i)}{\frac{1}{n_0}\sum_{i \in H_0} \frac{\hat{p}_0}{\hat{p}_{0|C_i}} \hat{S}_T(\tilde{t}|A = 0, \boldsymbol{M}_i, C_i)}$$

For each subject with $A = 0$ the hazard function is modelled to obtain a predicted estimate of the density and of the survival functions at time $\tilde{t}$ separately using the individual's own values of exposure, mediators and confounders.

Two weighted averages of these predicted values are computed for subjects with $A = 0$ (each subject *i* is given a weight $\frac{\hat{p}_0}{\hat{p}_{0|C_i}}$). The ratio of the two weighted averages is computed.

3. Estimation of $\lambda_{T^1,M^1}(\tilde{t})$:

$$Q_3(\tilde{t}) = \frac{\frac{1}{n_1}\sum_{i \in H_1} \frac{\hat{p}_0}{\hat{p}_{0|C_i}} \hat{f}_T(\tilde{t}|A = 1, \boldsymbol{M}_i, C_i)}{\frac{1}{n_1}\sum_{i \in H_1} \frac{\hat{p}_0}{\hat{p}_{0|C_i}} \hat{S}_T(\tilde{t}|A = 1, \boldsymbol{M}_i, C_i)}$$

It is computed as in 2., considering subjects with $A = 1$ and weights $\frac{\hat{p}_1}{\hat{p}_{1|C_i}}$. The probability estimates $\hat{p}_{0|C_i}$ and $\hat{p}_{1|C_i}$ in the denominator of the weights are always obtained by fitting suitable logistic regressions.

4. Computation of the effects: the pure direct effect, the total indirect effect and the total effect at time $\tilde{t}$ can then be obtained as follows:

$$PDE(\tilde{t}) = \frac{Q_1(\tilde{t})}{Q_2(\tilde{t})},$$

$$NIE(\tilde{t}) = \frac{Q_3(\tilde{t})}{Q_1(\tilde{t})},$$

$$TE(\tilde{t}) = PDE(\tilde{t}) \cdot NIE(\tilde{t}).$$

5. Computation of the confidence intervals (CI) of the effects: using non-parametric bootstrapping.

The procedure described above can be repeated for a given sequence of times $\tilde{t}$ thus allowing to observe how the causal effects possibly vary over time. Specifically we modelled the hazard function by the Royston-Parmar model (23, 24), a flexible parametric Cox model that estimates the baseline hazard using natural cubic splines. No constraints are imposed on the use of alternative survival models as long as they correctly specify the survival and the density functions.

All analyses were performed using the software R. We report in Web Appendix 2 the R code for the implementation of the estimation algorithm described above.

Simulations

We performed simulation studies to examine the finite sample performance of the proposed estimating procedure. We simulated a binary confounder *C* (1 with 50% frequency; 0 otherwise) and a binary exposure *A* (1 with 60% frequency in the group with *C*=1; and 1 with a 50% frequency in the group with *C*=0). We considered different scenarios with two mediators, M_1 and M_2. Firstly, we generated M_1 and M_2 according to the probabilities $P(M_1 = 1|A,C) = 0.15 + 0.25 \cdot A + 0.10 \cdot C$ and $P(M_2 = 1|A,M_1,C) = 0.20 + 0.30 \cdot A + 0.4 \cdot M_1 + 0.10 \cdot C$. In the second scenario, we replaced M_1 with a normally distributed variable according to the model

$M_1 = 0.15 + 0.25 \cdot A + 0.10 \cdot C + \varepsilon$, where $\epsilon \sim N(0, 0.4^2)$. In both scenarios we generated the time-to-event T according to the exponential model $\lambda_T(t|A, M_1, M_2, C) = e^{0.001} \cdot e^{\alpha \cdot A + \beta \cdot M_1 + \gamma \cdot M_2 + 0.10 \cdot C}$ and the censoring time from the exponential distribution with mean 1.25 (the censoring probabilities were 26-41%). Several configurations of the parameters α, β and γ were considered and for each configuration 500 simulated data sets were generated with a total sample size of n=2000.

The true causal effects for each data set were calculated by using equation (8) (Web Appendix 1). For each generated data set, the weighting approach was employed to estimate the causal quantities at the median survival time, and 95% CI were computed from 500 bootstrap samples. We considered the following simulation statistics: the average of the effect estimates, the standard deviation of the effect estimates, the bias and the coverage probability. Table 1 shows the simulation statistics for the case of two binary mediators while Table 2 shows the simulation statistics for the case of one binary and one continuous mediator. The bias ranges are (0.000-0.003) and (0.000-0.008) in the first and second scenario respectively. The coverage probabilities are equal or greater than 92% for both scenarios.

Empirical data example

In this Section we illustrate the methodology proposed using data from (25). In this paper the relationships among DNA methyltransferase genotype (polymorphism rs406193), DNA methylation, tumor aggressiveness (measured through Gleason score) and long-term mortality for prostate cancer were studied. It was hypothesized that rs406193 affected prostate-cancer mortality directly and indirectly via tumor tissue methylation and Gleason score. In fact it is known that DNA methylation is affected by the family of DNA methyltransferase enzymes, among which DNA methyltransferase 3b (DNMT3b) that was considered in the study. Furthermore in a

previous study (26) an association was found between tumor tissue DNA methylation in three selected genes (*GSTP1, APC, RUNX3*) and prostate cancer-specific mortality. It was hypothesized that: i) the activity of DNMT3b affects the methylation status of such genes; ii) their methylation status affects the Gleason score and not viceversa; iii) their DNA methylation affects prostate cancer mortality directly and indirectly through Gleason score (Figure 1). In mediation analysis terms, the exposure was the *DNMT3b* variant (carriers of at least one T compared to CC carriers), the two mediators were DNA methylation (coded with three levels: 0-1, 2 or 3 methylated genes respectively) and the Gleason score (coded with two levels, having or not a score ≥ 8) and the outcome was the time to death for prostate cancer. The *DNMT3b* variant and the DNA methylation were measured by analyzing DNA from slides of tumor tissue obtained at recruitment and also used to assign the Gleason score. The sequential temporality of the variables involved in the mediation pathway is plausible, being the *DNMT3b* variant time-independent, the DNA methylation an epigenetic process over time and the Gleason score a marker of tumo~~u~~r aggressiveness assumed to be affected by DNA methylation and not viceversa. Age at diagnosis, source for tumor tissue typing and period of diagnosis were considered potential confounders of exposure-outcome, mediators-outcome and exposure-mediators association.

The analyses of the reference paper highlighted clues on the role of genotype in prostate cancer mortality, however they did not decompose the total effect into direct and indirect effects. The study was based on 451 prostate cancer patients of any age diagnosed between 1982 and 1988 and between 1993 and 1996 at the San Giovanni Battista Hospital, Turin, Italy. Here we analyzed only subjects with complete information (n=393).

Having this example an educational purpose, our analyses focused on mortality from any cause in order not to consider the presence of competing risks (out of 333 observed events, 172 were deaths from prostate cancer and 161 from other causes). Doing so, we expect all the effects to be

diluted. Hence a further extension of the methodology to competing risks needs to be developed and it is the object of future research.

Firstly we carried out standard analyses to evaluate exposure-outcome, exposure-mediators, and mediators-outcome associations. Then we performed the mediation analysis using the weighting approach. We estimated the causal effects over about 100 equidistant epochs between the minimum and the maximum values of observed survival times. Confidence intervals were constructed using non-parametric percentile bootstrap.

By fitting a Royston-Parmar model adjusted only for confounders, T carriers had an hazard ratio of dying of 0.96 (95% confidence interval (CI): 0.77, 1.20). There was no evidence of association between carriers of the T allele and the number of methylated genes (adjusted odds ratio of each increase in the number of methylated genes = 0.84, 95% CI: 0.57, 1.23), while an association was found with Gleason score (adjusted odds ratio of having a score of 8 or more = 0.57, 95% CI: 0.39, 0.85). Moreover, there was an association between the two mediators (adjusted odds ratio of having ~~a~~ higher Gleason score = 1.45, 95% CI: 1.08, 1.94, the *DNMT3b* variant was considered among the covariates). Two Royston-Parmar models were fitted to estimate the associations between the two mediators and the outcome. Both models were adjusted for the exposure and the confounders. The model for Gleason score was also adjusted for DNA methylation. Subjects with 2 or 3 methylated genes had an increased risk of mortality compared to those with 0-1 methylated genes (adjusted hazard ratio: 1.25, 95% CI: 0.96, 1.61 for 2 versus 0-1 and 1.48, 95% CI: 1.08, 2.02 for 3 versus 0-1). For subjects with higher Gleason score the adjusted hazard ratio was 1.50, 95% CI: 1.20, 1.89. It is important to underline that the comparability of these estimates may be affected by non-collapsibility of hazard and odds ratios.

Figure 2 shows the causal effects estimated as a function of time. The PDE was close to the null value over the whole follow-up time (Figure 2A). There was evidence of a protective TIE only in the first years (Figure 2B). The TE showed an increasing pattern although there was no evidence

of a difference from the null value (Figure 2C). Table 3 shows the causal effects estimated at $\tilde{t} = 5$ and $\tilde{t} = 13$ years (approximately the median and the 95th percentile of the observed survival times). At 5 years from diagnosis, the TE was 0.98 (95% CI: 0.81, 1.18), the TIE 0.96 (95% CI: 0.91, 1.02) and the PDE 1.02 (95% CI: 0.85, 1.21). At 13 years, the TE was 1.07 (95% CI: 0.90, 1.28), the TIE was 1.06 (95% CI: 0.97, 1.18) and the PDE was 1.01 (95% CI: 0.89, 1.15).

The positivity assumption was checked (27, 28) and the analyses did not suggest violations (range of the estimated propensity score: 0.24, 0.60). We assumed the absence of unmeasured confounders of exposure-outcome, exposure-mediators, mediators-outcome associations and the absence of an effect of the exposure that confounds mediators-outcome relationship. However the estimates of PDE and TIE and, hence, of the TE could be biased by the presence of some unmeasured mediator-outcome confounders such as possible non-epigenetic molecular signatures pointing toward Gleason score and mortality.

To explore the role of single mediators, we conducted an additional analysis including only DNA methylation. The models with and without Gleason score may be not directly comparable because of non-collapsibility of hazard ratio. However if this is assumed not to affect greatly the estimates and the models' aptness, this analysis may suggest how the addition of Gleason score modifies the effect estimates. Figure 3 and Table 4 report the estimated effects. Similarly to the results obtained previously, PDE was always close to the null value (Figure 3A) and the TIE showed a protective effect only in the first years, although further attenuated (Figure 3B). The PDE now captures also the effect of the genetic variant on mortality through the Gleason score independently from DNA methylation, and the TIE incorporates all the pathways through DNA methylation.

Discussion

In this article we have introduced a procedure to estimate pure direct and total indirect effects through multiple mediators in a survival setting by showing how to extend the weighting approach proposed in (13) to survival outcomes.

Few methods have been introduced in literature for multiple mediation analysis with survival data. A simple approach that can be used with any generalized linear model was developed in (14). Such method has the advantage of overcoming the need to specify possible exposure-mediators interactions and it can be implemented with standard software (29). However difficulties may arise in detecting small indirect effects. In (15) a weighting approach for multiple mediation was proposed that can be used for most types of outcomes. It requires distinct causal pathways for the mediators and shows a worse performance in the case of continuous mediators. Despite being a weighting approach, the estimation procedure requires besides a model for the exposure also a model for each mediator in the construction of the weights. In (17) another multi-mediator model was devised specifically for survival data with continuous mediators and a continuous or binary exposure. Its main advantage is that it allows the examination of path-specific effects of each mediator. However it is employable only in a low-dimensional setting (one or two mediators). More recently, in (18) methods for multi-mediator analyses have been proposed using Aalen models, Cox models with rare outcomes and semiparametric probit models. Closed-form expressions for path-specific effects are provided requiring models for the mediators with normal errors. In (19) an approach to estimate interventional analogues of direct and indirect effects through a survival mediational g-formula is developed. The approach can be used with time-varying exposures, mediators, and confounders. However the outcome only focuses on survival probability at the end of follow-up and the extension to different survival models is proposed as a future perspective. Similarly, in (20) the effect of a time-varying exposure mediated by a time-varying mediator is studied in a survival setting, proposing a formulation in terms of random interventions. Three different double robust semi-parametric efficient estimators are presented, among which one based on inverse probability weighting.

The approach described in the present paper allows the estimation of marginal causal effects on the hazard function scale. The estimation performance is highly dependent on the validity of its assumptions among which the correct specification of the model for the outcome. In particular we used a flexible parametric Cox model, though alternative survival models including accelerated failure time and additive hazards models could be applied. Furthermore, the methodology needs to be extended in order to model appropriately ~~the~~ competing risks. Finally a sensitivity analysis should be performed to test the unmeasured confounding assumptions, but further studies are still needed. To our knowledge, Tchetgen Tchetgen and Shpitser (30) proposed a semiparametric sensitivity analysis technique for the presence of unmeasured confounding on the estimation of the effects for several types of outcomes, including common survival ones. Our aim is to extend it in the near future to take into account multiple mediators simultaneously as well as the time-dependent effects.

The method has been implemented under the assumption of proportionality for the conditional hazard functions and it can be applied in the presence of both non-rare and rare outcomes, with estimated marginal effects respectively varying or nearly constant over time. The causal mediation effects on the hazard function scale are estimated over a grid of times. For this aspect, the method is similar to that proposed in (11), but has the advantage of being applicable with multiple mediators of any nature, also not normally distributed. It allows exposure-mediators interactions and interactions between mediators and it does not require models for the mediators nor their independence.

A limitation is its inability to characterize the path-specific effects of each mediator (31). Several procedures have been proposed in literature under various settings (16, 32-35) but few explicitly for survival analysis (17, 18). Since proportional hazards models are commonly used in biomedical research, the development of methodologies enabling to incorporate multiple mediators and to characterize path-specific effects is an important direction for future research.

Our procedure requires the computation of weights, which are particularly sensitive to bias due to data sparsity. Bias can arise due to positivity violations or because some confounders-exposure combinations are not represented or under-represented in the finite sample by chance. Diagnosis and quantification of this bias is recommended (27, 28, 36). Finally this approach can be only used with binary or categorical exposures. In fact, although the paper primarily focuses on binary exposures, the approach equally applies for categorical ones considering a fixed reference category and estimating the causal effects for each of the others with respect to that one.

The main contribution of this paper is to give a useful tool in mediation analysis in the presence of multiple mediators and survival outcomes. The proposed approach involves probability weights that relate exposure, mediators and confounders and therefore can be implemented in most standard software.

Acknowledgements section

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Conflict of interest statement

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Figure legends

Figure 1: Direct acyclic graph representing the assumed causal relationships between *DNMT3b* (*A*), DNA methylation in *APC, GSTP1, RUNX3* (M_1), Gleason score (M_2), and prostate cancer mortality (*T*). ***C*** is the vector of confounders: age at diagnosis, source for tumor tissue typing and period of diagnosis.

Figure 2: Plots of causal effects (PDE in Figure 2A, TIE in Figure 2B, TE in Figure 2C) of genetic variant on mortality as a function of time, considering DNA methylation and Gleason score as mediators.

Figure 3: Plots of causal effects (PDE in Figure 3A, TIE in Figure 3B, TE in Figure 3C) of the effect of genetic variant on mortality as a function of time, considering only DNA methylation as mediator.

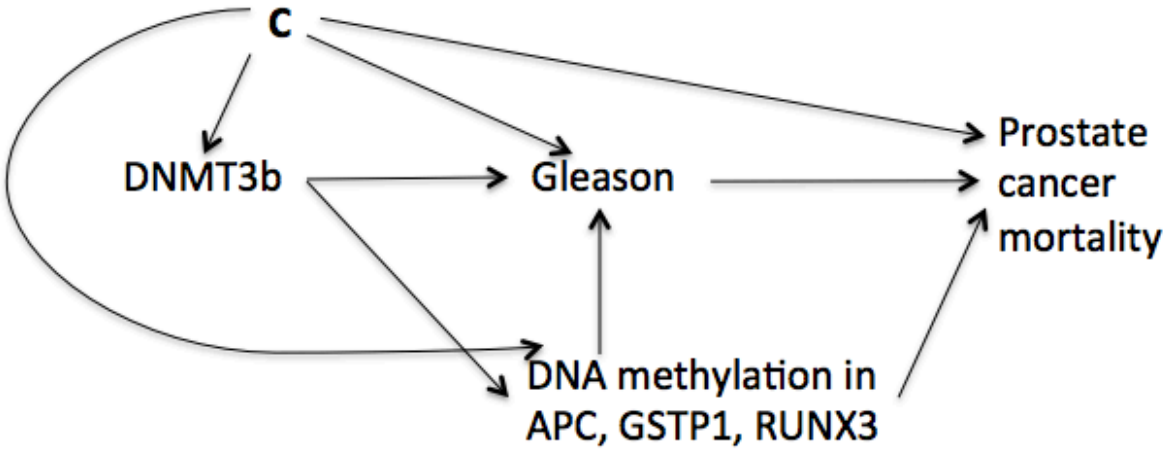
C
DNMT3b
Gleason
Prostate cancer mortality
DNA methylation in APC, GSTP1, RUNX3

Figure 1

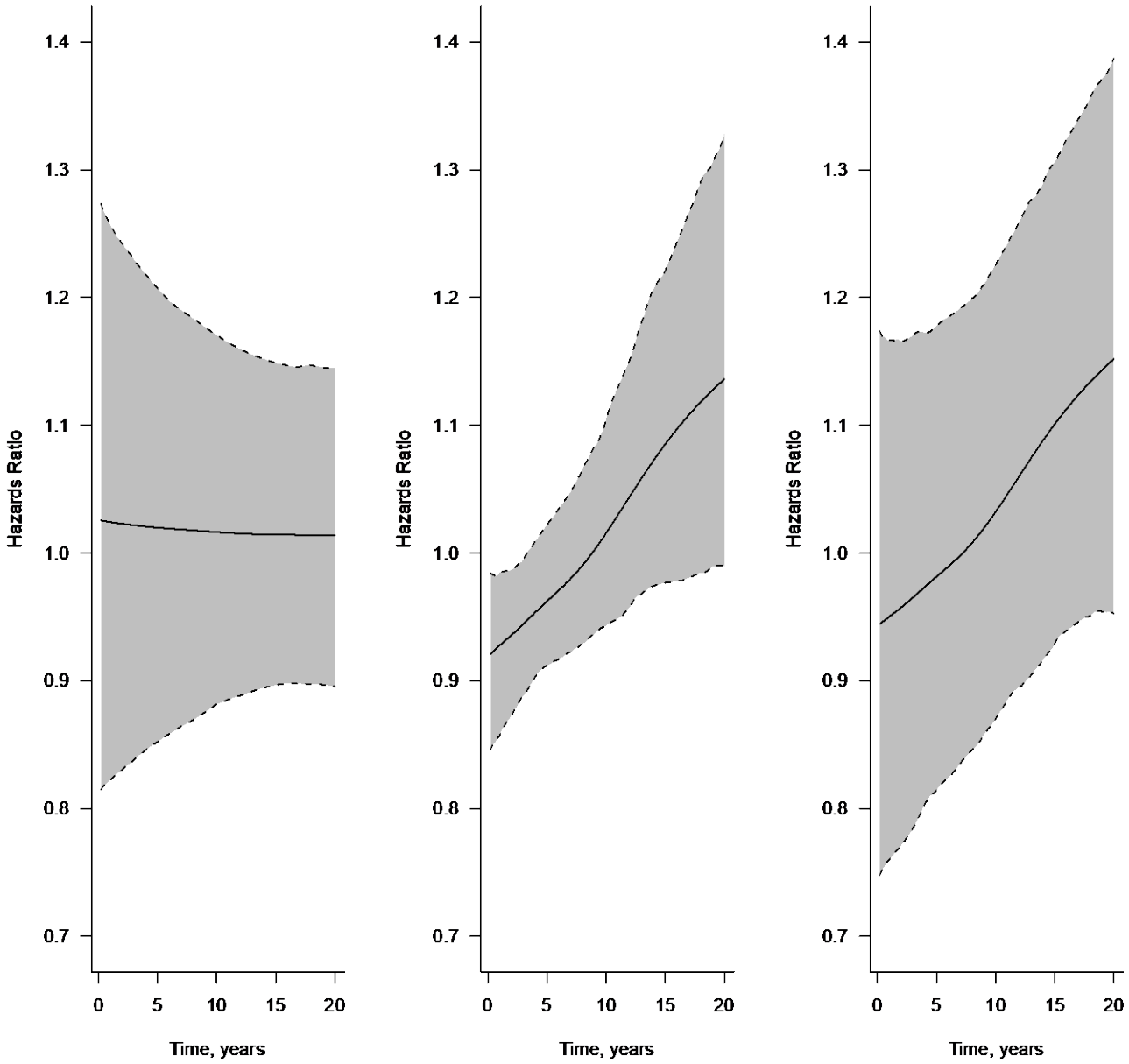

Figure 2

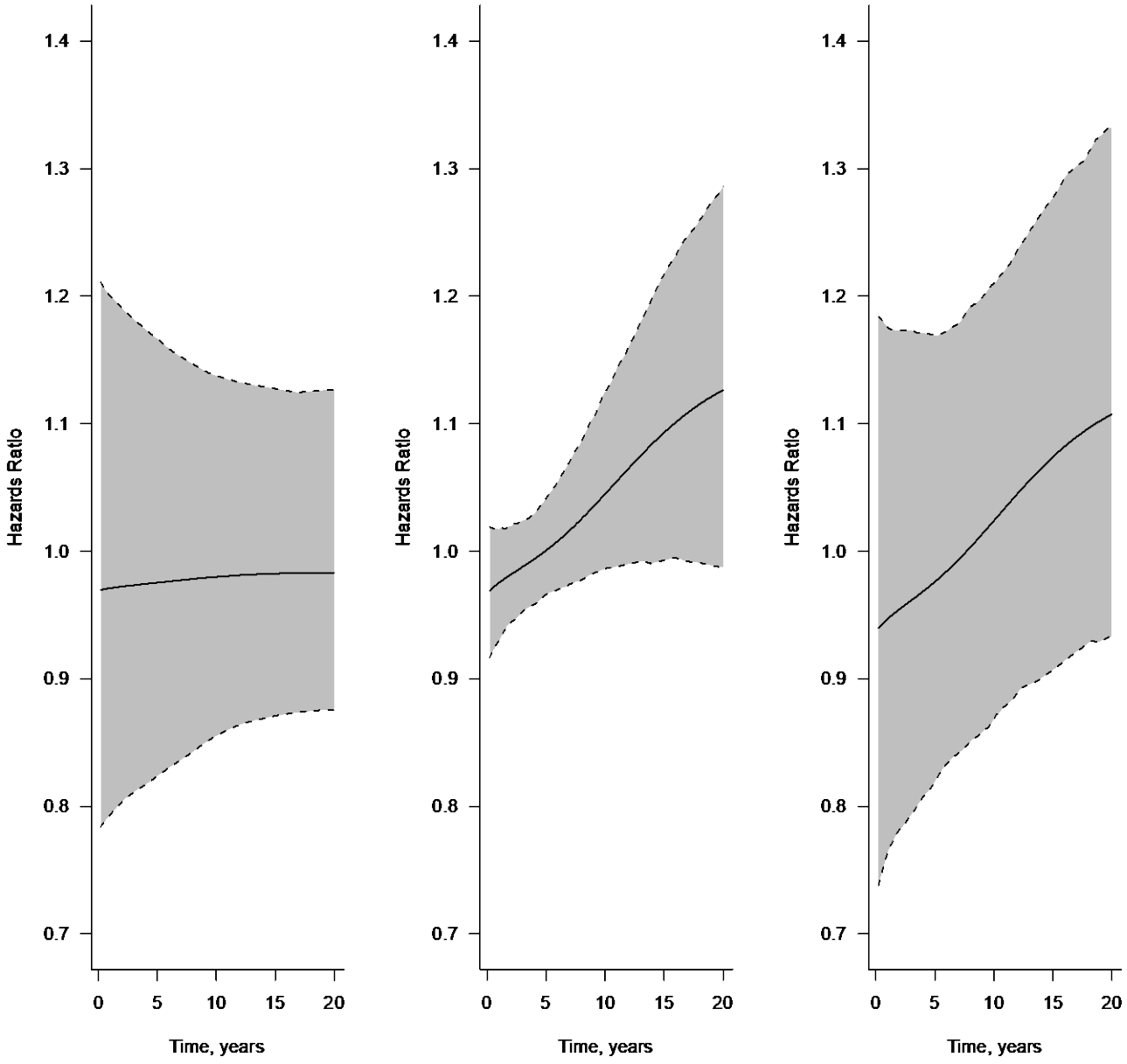

Figure 3

Table 1: Simulation statistics for the estimated effects in the first scenario (two binary mediators).[a]

α	β	γ	PDE($\tilde{t}$)				TIE($\tilde{t}$)				TE($\tilde{t}$)			
			True	Mean (sd)	Bias	Cov Prob	True	Mean (sd)	Bias	Cov Prob	True	Mean (sd)	Bias	Cov Prob
0.05	0.6	0.7	1.044	1.042 (0.05)	-0.002	0.96	1.473	1.476 (0.04)	0.003	0.95	1.538	1.536 (0.08)	-0.002	0.94
0.5	0.06	0.07	1.645	1.644 (0.10)	-0.001	0.95	1.043	1.044 (0.03)	0.003	0.95	1.716	1.715 (0.10)	-0.001	0.95
0.05	0.06	0.07	1.051	1.049 (0.07)	-0.002	0.96	1.044	1.045 (0.03)	0.001	0.94	1.097	1.096 (0.06)	-0.001	0.95
0.5	0.6	0.7	1.545	1.543 (0.08)	-0.002	0.96	1.459	1.461 (0.04)	0.002	0.95	2.254	2.254 (0.12)	0.000	0.95
0.5	0.06	0.7	1.590	1.589 (0.09)	-0.001	0.96	1.338	1.340 (0.04)	0.002	0.96	2.127	2.128 (0.12)	0.001	0.94
0.5	0.6	0.07	1.610	1.608 (0.09)	-0.002	0.97	1.171	1.172 (0.03)	0.001	0.94	1.886	1.884 (0.10)	-0.002	0.95

Abbreviations: pure direct effect (PDE), total indirect effect (TIE), total effect (TE), standard deviation (sd), coverage probability (Cov prob).

[a]For each configuration of the parameters the true value of the effects (true), the mean and the standard deviation of the estimates obtained on 500 simulated datasets, the bias (difference between the true value and the average estimate) and the coverage probability (the percentage of simulated data sets for which the 95% confidence interval for estimated causal quantities covered the true value) are reported. The effects are assessed at the median survival time $\tilde{t}$.

Table 2: Simulation statistics for the estimated effects in the second scenario (a continuous and a binary mediators).[a]

α	β	γ	PDE($\tilde{t}$)				TIE($\tilde{t}$)				TE($\tilde{t}$)			
			True	Mean (sd)	Bias	Cov Prob	True	Mean (sd)	Bias	Cov Prob	True	Mean (sd)	Bias	Cov Prob
0.05	0.6	0.7	1.044	1.047 (0.06)	0.003	0.94	1.488	1.488 (0.04)	0.000	0.96	1.554	1.557 (0.08)	0.003	0.95
0.5	0.06	0.07	1.646	1.654 (0.11)	0.008	0.94	1.044	1.045 (0.03)	0.001	0.96	1.718	1.726 (0.10)	0.008	0.95
0.05	0.06	0.07	1.052	1.055 (0.07)	0.003	0.94	1.044	1.045 (0.03)	0.001	0.97	1.097	1.102 (0.06)	0.005	0.94
0.5	0.6	0.7	1.551	1.557 (0.09)	0.006	0.92	1.482	1.482 (0.04)	0.000	0.95	2.298	2.306 (0.13)	0.008	0.94
0.5	0.06	0.7	1.590	1.596 (0.10)	0.003	0.93	1.339	1.336 (0.04)	-0.003	0.95	2.129	2.131 (0.13)	0.002	0.93
0.5	0.6	0.07	1.614	1.620 (0.10)	0.006	0.94	1.183	1.185 (0.03)	0.002	0.96	1.919	1.919 (0.11)	0.000	0.94

Abbreviations: pure direct effect (PDE), total indirect effect (TIE), total effect (TE), standard deviation (sd), coverage probability (Cov prob).

[a]For each configuration of the parameters the true value of the effects (true), the mean and the standard deviation of the estimates obtained on 500 simulated datasets, the bias (difference between the true value and the average estimate) and the coverage probability (the percentage of simulated data sets for which the 95% confidence interval for estimated causal quantities covered the true value) are reported. The effects are assessed at the median survival time $\tilde{t}$.

Table 3: Causal effects at times $\tilde{t}$ = 5 (median) and $\tilde{t}$ = 13 (95th percentile) years considering DNA methylation and Gleason score as mediators.

Estimated effects	***DNMT3b* rs406193**		
	CC carriers	CT+TT	95% CI
PDE($\tilde{t}$ = 5)	1	1.02	0.85, 1.21
TIE($\tilde{t}$ = 5)[a]	1	0.96	0.91, 1.02
TE($\tilde{t}$ = 5)	1	0.98	0.81, 1.18
PDE($\tilde{t}$ = 13)	1	1.01	0.89, 1.15
TIE($\tilde{t}$ = 13)[a]	1	1.06	0.97, 1.18
TE($\tilde{t}$ = 13)	1	1.07	0.90, 1.28

Abbreviations: confidence interval (CI), carriers of at least one T (CT+TT), pure direct effect (PDE), total indirect effect (TIE), total effect (TE).

[a]through DNA methylation and Gleason score

Table 4: Causal effects at times $\tilde{t}$ = 5 (median) and $\tilde{t}$ = 13 (95th percentile) years considering only DNA methylation as mediator.

Estimated effects	***DNMT3b* rs406193**		
	CC carriers	CT+TT	95% CI
PDE($\tilde{t}$ = 5)	1	0.98	0.82, 1.17
TIE($\tilde{t}$ = 5)[a]	1	1.00	0.97, 1.04
TE($\tilde{t}$ = 5)	1	0.98	0.82, 1.17
PDE($\tilde{t}$ = 13)	1	0.98	0.87, 1.13
TIE($\tilde{t}$ = 13)[a]	1	1.07	0.99, 1.18
TE($\tilde{t}$ = 13)	1	1.05	0.90, 1.25

Abbreviations: confidence interval (CI), carriers of at least one T (CT+TT), pure direct effect (PDE), total indirect effect (TIE), total effect (TE).

[a] through only DNA methylation