Research Methods

Comparative Effectiveness Study in Multiple Sclerosis Patients Using Instrumental Variable Analysis

Hamed Hosseini, MD[1]; Mohammad Ali Mansournia, MD[1]; Seyed Massood Nabavi, MD[2]; Ali Asghar Akhlaghi, PhD[3]; Jaleh Gholami, MD[4]; Kazem Mohammad, PhD[1]; Reza Majdzadeh, PhD[1,5]

[1]Department of Epidemiology and Biostatistics, Public Health School, Tehran University of Medical Sciences, Tehran, Iran
[2]Neurology Group, Department of Regenerative Biomedicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
[3]Department of Epidemiology and Reproductive Health, Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran,
[4]Iranian National Center for Addiction Studies, Tehran University of Medical Sciences, Tehran, Iran
[5]Knowledge Utilization Research Centre (KURC), Tehran University of Medical Sciences, Tehran, Iran

Abstract
Randomized clinical trials are considered the ideal source for generation of robust evidence for clinical and public health decision making. Estimation of treatment effect in observational studies is always subject to varying degrees of bias due to lack of random allocation, blindness, precise definition of intervention, as well as the existence of potential unknown and unmeasured confounding variables. Unlike other conventional methods, instrumental variable analysis (IVA), as a method for controlling confounding bias in non-randomized studies, attempts to estimate the treatment effect with the least bias even without knowing and measuring the potential confounders in the causal pathway. In this paper, after understanding the main concepts of this approach, it has been attempted to provide a method for analyzing and reporting the IVA for clinical researchers through a simplified example. The data used in this paper is derived from the clinical data of the follow-up of multiple sclerosis (MS) patients treated with two class of interferon.
Keywords: Clinical trials, Multiple sclerosis, Patients, Variable analysis
Cite this article as: Hosseini H, Mansournia MA, Nabavi SM, Akhlaghi AA, Gholami J, Mohammad K, et al. Comparative effectiveness study in multiple sclerosis patients using instrumental variable analysis. Arch Iran Med. 2018;21(8):368–375.

Introduction

Practitioners and patients are always looking for the most effective treatment among different alternatives.[1] Although the randomized clinical trials provide robust evidence for decision making, a head-to-head comparison of treatments is not feasible due to resource constraints, timeliness, competitive considerations of manufacturing companies, regulatory affairs, market forces, and patients' preferences.[2] Moreover, rare side effects of medications and long-term effects of treatments require a much longer follow-up than the duration of clinical trials.[3]

In the real world, health systems are bound to use administrative data for timely decision-making, while the data are mostly collected with non-research objectives and exposed to different types of bias.[4] Comparative effectiveness research (CER) aimed at achieving a better clinical decision,[4,5] performed through a variety of ways such as restriction, matching,[6] stratification, regression models, propensity score method including inverse probability of treatment weighting,[7–9] standardization,[10] and g-estimation[11,12] try to control various confounding sources. In all the aforementioned, potential confounding factors should be well known and measured. However, with all these attempts, the residual bias and unmeasured or unknown confounders' effects are not fully overcome.[13,14] This happens when we know that lack of the unmeasured confounder effect on causal assessment is an essential assumption in conventional statistical methods.

To address the problem of unknown and unmeasured confounders in non-randomized studies, instrumental variable (IV) analysis has been introduced as an unbiased estimation of treatment effect (even in the presence of unmeasured confounders).[3] Given the increasing use of this method in biomedical research, this paper attempts to provide the readers with the most important prerequisites to achieve this goal in a simple manner using a real example.

Instrumental Variable Analysis
Instrumental variable analysis (IVA) is a method for controlling the effect of unmeasured confounders in

*Corresponding Author: Reza Majdzadeh, PhD; Department of Epidemiology and Biostatistics, Public Health School, Tehran University of Medical Sciences, Tehran, Iran. Address Department of Epidemiology and Biostatistics, Public Health School, Tehran University of Medical Sciences, Tehran, Iran. Tel: +98-21-42933333, Email: rezamajd@tums.ac.ir

non-randomized studies. This method begins by finding a variable that only influences the outcome through the treatment pathway that is independent of the confounding factors. Then, this variable is used to estimate the changes of treatment effect, without being affected by the confounding effects. Finally, the causal effect of treatment is estimated with variances independent of the confounding effect. In fact, this method tries to achieve a low bias estimation of treatment effect on the clinical outcome by converting the observational data into a randomized clinical trial.[13–15]

An IV has 3 main attributes (Figure 1). The first is the relationship between the instrument (Z) and the treatment X (relevance) that determines which people receive the treatment. As in the clinical trial, the random allocation process assigns the participants to different treatment groups. The second feature, or the effective random assignment (ERA), states that the instrument is independent of the known or unknown confounding variables (U). As in an ideal randomization, individuals are assigned to therapeutic groups, independently, without considering known or unknown confounders. The third feature, or exclusion restriction (ER), states that the instrument has no effect on the treatment outcome (Y) from any other pathway, except the allocated treatment one (X). Since it is not always possible to find such a variable, the conditional IV (Z*) (i.e., the variable that can play the role of an IV through controlling the other known variables) is mostly used.[14–16]

1- Step One: Selection of the Instrumental Variable

Before deciding to use an IVA, it is necessary to make sure whether the impact of the set of unmeasured confounders on the outcome is important enough to distort the estimation of efficacy and clinical decision-making. If the unmeasured confounders are important, IVA can be a logical approach.[15] Various IVs are used including randomized encouragement, calendar times, provider preferences, geographic distance, and insurance plan.[17,18] Regardless of the type of IV, it is necessary to always check the appropriateness of IV assumptions before the final analysis (Figure 1). The important assumptions of an IVA are:

1.1. IV and Intervention Relationship (Relevance Assumption)

For IVA to control the effect of potential confounders, a strong relationship is necessary between the IV and the intervention such that the IV can be a strong predictor of intervention. The stronger the relationship, the more capability on the part of the IVA to eliminate the confounding effect.[3] To this end, the following methods are used simultaneously:
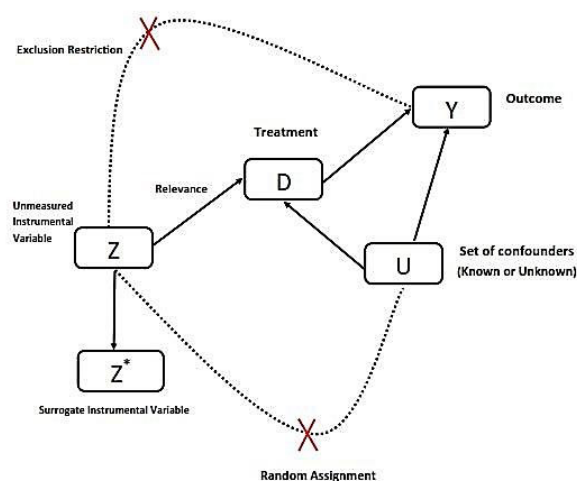
Figure 1. An Illustration of Instrumental Variable Analysis and Core Assumptions.

1.1.1. Calculation of the complier proportion: This proportion is obtained by calculating the difference in the treatment assignment rate in different layers of IV, and the larger complier proportion indicates the effective sample size in controlling the confounding effects in the IVA.

1.1.2. Calculation of F-statistics (degree of freedom=1) for the IV in a regression model in which the intervention variable is entered as the dependent and other covariates as independent variables (In fact, the inequality test of α1 with zero in Equation 1).

1.1.3. Partial R square in the regression model in which the intervention variable is entered as the dependent variable and the IV and other measured confounders as independent variables; this number actually represents the unexplained variance percentage by the covariance measured in the first step of IVA that is expected to be explained by IV.[14,15,18]

1.2. IV Independence of Unmeasured Confounders (ERA Assumption)

Although this assumption cannot be tested statistically, the balanced distribution of potential measured confounders in different layers of the IV can greatly depict its correctness[3,8]; the same role is played by the table of baseline characteristics among the comparison groups in a clinical trial. For this reason, a calculated standardized difference of more than 0.2 in the comparative therapeutic groups and 0.2 multiplied by complier rate in the layers of IV indicates the existence of an imbalance.[3]

1.3. Exclusion Restriction Assumption

This assumption implies the effect of an IV on the outcome is only through the intervention. However,

during the treatment process, patients receive a variety of medicinal/non-medicinal recommendations from their physicians in addition to medication, which can directly or indirectly affect the patients' outcomes (potential confounder). Investigating the relationship between an IV and such variables can somewhat help to ensure the ER assumption.[3,15]

2. Step Two: Estimation of the Treatment Effect

The IVA uses the linear structural equation modeling technique to examine the causal link, rather than the association. In this model, most of the two-stage least square (2SLS) estimator measures the treatment efficacy in the form of a risk difference. In the first stage, the intervention variable (X) is selected as the dependent variable of the regression model, and the IV (Z or Z*) and the measured covariate sets are selected as independent variables (Figure 2). Simply put, the most important use of this step is actually to calculate the predicted probability of the allocation of people into different treatment groups given by IV and the set of confounders.

In the second stage, the outcome variable (Y) is used as the dependent and the predicted value of the treatment variable in terms of the IV and selected covariates (obtained from the first stage) as independent variables. Therefore, the coefficient of the treatment variable in the second model, IV estimator, is the estimate of the causal effect of the treatment.[2,18]

3. Step Three: Sensitivity Analysis

It is not always possible to find an ideal IV, and the extent of the violation of the mentioned assumptions should be obtained using a sensitivity analysis method.

3.1. Sensitivity Analysis for Violation of the Effective Random Assignment assumption

Contrary to this assumption, consider that there is a set of unknown confounders, the effects of which we have not been able to control. Assume, without loss of generality, this unmeasured confounder has a distribution with a mean of zero and standard deviation of 1 (standardized distribution), and 1 standard deviation increase in hypothetical confounder resulted in δ change on the outcome. Also, the effect of IV on this assumed confounder is called τ.

So, the sensitivity analysis will be affected by δ and τ as two tuning parameters. Now, if expected changes in these two hypothetical parameters resulted in no clinically important change in estimated effect by the IV model (especially in terms of direction), the results of the IVA can be largely trusted.
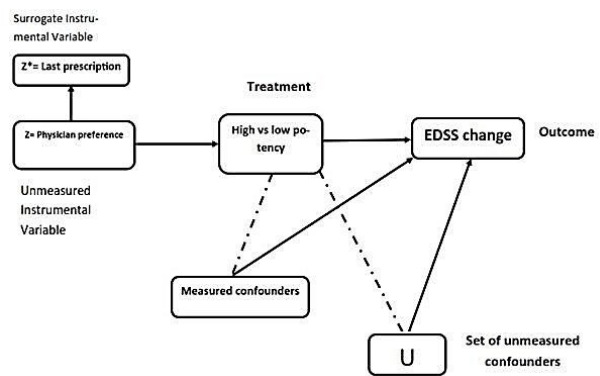

Figure 2. Effect of Interferon Class on EDSS Change.

3.2. Sensitivity Analysis for Violation of the Exclusion Restriction Assumption

It has already been stated that the effect of the IV on the outcome is assumed to transfer only through the intervention. Now, imagine that practitioners with high potency preference (IV = 1), may measure Expanded Disability Status Scale (EDSS) better than those with low potency preference (IV = 0). Thus, the selected IV affects the outcome in a way other than the treatment (violation of the ER assumption). To ensure non-violation, the λ parameter is defined the treatment effect modification or the difference in the size of the effect in the layers of IV. This parameter actually is the coefficient of the interaction term between the treatment and the IV in the outcome model.[3,15]

Case Study

In order to provide a practical example of IVA use, data from patients with multiple sclerosis (MS) are provided in this section. These data are about prescribing low/high potency interferon beta-1a in patients with relapsing-remitting multiple sclerosis (RRMS) from October 2011 to October 2016 by 18 neurologists in Tehran.

As a chronic inflammatory neurodegenerative disease, MD requires lifelong treatment with immunosuppressants, immunomodulators, and monoclonal antibodies.[19] RRMS initially manifests itself with a neurological attack, and often the patient completely recovers from the initial symptoms within a short time interval after the attack even though subclinical lesions may remain or even progress. In effective immunomodulatory treatment, which begins immediately after the onset of the first symptoms, it is expected not only to delay the second demyelinating event but also to prevent permanent disability.[20,21] As of 2018, 15 drugs have been approved by the U.S. FDA for modifying the course of MS including 5 interferon beta,[15] which are recommended as first-line therapy in RRMS. Although some head-to-head comparison between low-dose and low-frequency

interferon beta-1a and subcutaneous high-dose and high-frequency interferon beta-1a and interferon beta1b have revealed that high-dose and high-frequency interferon beta regimens have short-term benefits on the relapse rate and MRI activity, there are limitations in the design of these studies and the long-term differences in efficacy are not clearly concluded.[22]

In drug therapy studies of MS patients, various outcomes are measured. For simplicity and applicability of the example, in this study, changes in EDSS was selected as the outcome variable. EDSS is a common criterion for assessing the incapacity in MS patients and is calculated by the physician based on examination of 8 functional systems. It is used to classify the severity of MS, the rate of disease improvement or progression, the rate of disability, and evaluation of treatment outcomes. The range of changes in EDSS is zero (no disability) to 10 (death).

In this study, the compared interventions included injection of high potency drugs such as subcutaneous injection of 250 μg betaferon (interferon beta-1b) per day versus the treatment efficacy of low potency drugs such as intramuscular injection of 30 μg Avonex per week and its impact on the EDSS was measured over a year.

Results

The clinical records of 290 patients with RRMS were investigated during the period of October 2011 to October 2016. From the total of 290 patients, 141 (48.62%) were in the low potency group and 149 (51.38%) were in the high potency group. The mean (SD) age of the low potency and the high potency groups was 31.99 (8.45) and 33.92 (8.69) years, respectively. 115 subjects (82.14%) in the high potency group and 109 subjects (73.15%) in the high potency group were females (Table 1).

The results of linear regression analysis without adjustment and following adjustment to baseline EDSS, duration of disease, delay in diagnosis, relapse number showed that the difference in the treatment efficacy of the high potency group compared to the low potency group was 0.27 and 0.40, respectively (Table 2).

Step 1: Selection of an Instrumental Variable

Various reasons, such as the physician's clinical evaluation method, the patient's financial condition, preferences, and insurance coverage are effective in prescribing a specific drug by physicians. These characteristics are often associated with clinical outcomes of the patient and are often not measured or reported in clinical records. For this reason, the direct comparison of the results of different treatments leads to an incorrect estimation of the efficacy because of unmeasured confounders in the data. In such a situation, the IVA can help in correct estimation of treatment efficacy.

In this study, physician's preference was used as the IV. The preference-based variables class is often used in clinical settings.[17,23,24] For this purpose, we ranked all patients according to date of the first prescription. Then, the immediately previous prescription of the physician was selected as the preferred physician's variable at the next prescription. Therefore, the selected IV is a binary variable, and since the actual preference of the physician (Z) is not directly measurable, the preferred variable has been chosen based on the previous prescription (Z *) (Figure 2).

By choosing this IV, there are actually 3 main assumptions; the prescription preference variable is associated with placing the patient in the low potency or high potency group (first assumption), no difference exists in the distribution of patient characteristics in different layers of the preferred prescription variable after adjusting to the measured confounding variables (second hypothesis), and the quality of treatment among physicians with different preference is the same (third presumption).

Investigating the Relationship Between IV and Interference (Relevance Assumption)

To calculate the complier proportion, the difference in the percentage of high potency recipients was calculated

Table 1. Imbalance of Measured Potential Confounders Between Low Potency Versus High Potency Treatment

Measured Covariates	Treatment Group				IV Preference				
	Low Potency	High Potency	P Value	StD	Low Potency	High Potency	P Value	StD	Bias
Female (%)	115 (82.14)	109 (73.15)	0.07	0.22	121 (80.67)	103 (74.10)	0.18	0.16	1.02
Age (SD)	31.99(8.45)	33.92(8.69)	0.06	0.22	32.51(8.52)	33.50 (8.70)	0.33	0.11	0.71
Cousin marriage (%)	19 (13.77)	33 (22.15)	0.07	0.22	28 (18.92)	24 (17.27)	0.72	0.04	0.27
Disease duration in month (SD)	61.70(57.53)	80.99(71.76)	0.01	0.30	69.64 (63.91)	73.97 (68.16)	0.58	0.07	0.31
Diagnosis delay in month (SD)	18.68(12.65)	19.15(39.16)	0.93	0.02	17.88(35.02)	20.75(40.45)	0.59	0.08	8.48
Relapse (median)	0.85 (1)	1.6 (1)	0.00	0.44	0.99 (1)	1.51 (1)	0.01	0.30	0.96
Baseline EDSS (SD)	1.59 (1.34)	2.43 (1.51)	0.00	0.58	1.89 (1.63)	2.17 (1.51)	0.12	0.19	0.46
Multifocal lesions (%)	46 (33.33)	62 (41.61)	0.15	0.18	56 (51.9)	52 (48.15)	0.94	0.01	0.63

Abbreviations: StD, standardized difference; SD, standard deviation; EDSS, Expanded Disability Status Scale.
Bias Ratio= ((AGE/IV=1)-(AGE/IV=0) / (GROUP=1/IV=1)-(GROUP=1/IV=0)) / ((AGE/GROUP=1)-(AGE/GROUP=0)).

Table 2. Estimating the Effect of CD Versus AB on EDSS Change in RRMS

	β	SE	95% CI
Regression 1	0.27	0.10	0.07–0.47
Regression 2	0.28	0.11	0.07–0.49
Regression 3	0.39	0.11	0.18–0.60
Regression 4	0.26	0.10	0.05–0.46
Regression 5	0.42	0.11	0.20–0.63
Regression 6	0.40	0.12	0.18–0.62
IV regression 1	0.02	0.13	-0.24–0.29
IV regression 2	0.01	0.14	-0.26–0.29
IV regression 3	0.17	0.14	-0.11–0.46
IV regression 4	0.01	0.13	-0.25–0.28
IV regression 5	0.01	0.14	-0.26–0.28
IV regression 6	0.19	0.14	-0.10 - 0.48

Abbreviations: CI, confidence interval, SE, dtandard error.
Regression 1: Standard regression approach which estimates the effect of CD versus AB on EDSS change
Regression 2: Standard regression approach which estimates the effect of CD versus AB on EDSS change including relapse Number into a regression model
Regression 3: Standard regression approach which estimates the effect of CD versus AB on EDSS change including diagnosis delay into a regression model
Regression 4: Standard regression approach which estimates the effect of CD versus AB on EDSS change including duration of disease into a regression model
Regression 5: Standard regression approach which estimates the effect of CD versus AB on EDSS change including relapse Number and diagnosis delay into a regression model
Regression 6: Standard regression approach which estimates the effect of CD versus AB on EDSS change including relapse Number, diagnosis delay, and duration of disease into a regression model
IV Regression 1: 2SLS IV analysis not includes any covariates.
IV Regression 2: 2SLS IV analysis not including relapse Number as covariate.
IV Regression 3: 2SLS IV analysis not including diagnosis delay as covariate.
IV Regression 4: 2SLS IV analysis not including duration of disease as covariate.
IV Regression 5: 2SLS IV analysis not including relapse Number and diagnosis delay as covariates.
IV Regression 6: 2SLS IV analysis not including relapse Number, diagnosis delay, and duration of disease as covariates.

in layers of IV groups (high potency preference group versus low potency preference group) and resulted in a proportion of 71.82%. The results of the Durbin and Wu-Hausman tests (tests of endogeneity, which examines the assumption of variable exogeneity), indicated that the intervention variable actually has the conditions of an endogenous variable ($P = 0.0432$). Moreover, the F-statistic index calculated as 186.05 in the regression model (including the intervention variable as the dependent variable and other covariates as independent variables) showed a proper correlation (F > 10). The partial R square calculated in the model (percentage of unexplained variance by the measured confounders in the first stage of IVA which is expected to be explained by IV) was 52%, which is expected to be controlled by the IV in the second stage.

Evaluation of IV Independence From Unmeasured Confounders (Effective Random Assignment)
The distribution of confounding variables in the layers of IV greatly showed that the distribution of these

variables in the IV layers was homogeneous compared to the treatment group layers (endogenous variable) (Table 1) although this imbalance is still observed, for example, in the recurrence variable. Moreover, as shown in Table 1, the calculated bias ratio (bias value from the non-controlling effect of X-factor in IVA compared to OLS approach) showed that in values less than one, the IV method is more optimal than the usual OLS method in the control of bias.[14]

Evaluation of Exclusion Restriction Assumption
When an IV is associated with concomitant therapy, which affects the outcome, the ER assumption is violated. For this purpose, we explored patient treatment profiles completely and did not find any co-treatment among them. Based on our judgment, we are convinced the IV only influence the EDSS through its association with the treatment in this study and it satisfies the ER assumption.

Step 2: Analysis of Instrumental Variable and Estimation of Treatment Effect Difference
It is revealed that the EDSS change between the high potency treatment group compared to the low potency recipients was reduced from 0.40 (95% CI: 0.18-0.62) in OLS estimation to 0.19 (95% CI: -0.10-0.48) in the IVA.

Step 3: Sensitivity Analysis
Sensitivity analysis for ERA assumption
It is assumed there is an unknown confounder, such as "delay in the diagnosis; a significant measured confounder in the study", that is not related to the measured confounders but is related to the IV (violation of the ERA assumption). The standardized residual has been calculated in the regression model with "delay in the diagnosis" as the outcome and other confounders as the predictors. Then, the relationship between this hypothetical confounder with the outcome (δ) and with the IV (τ) was estimated -0.001 and 0.10, respectively (Table 3).

The same process, assuming that there is a confounding variable such as the baseline EDSS, was repeated and the sensitivity parameters δ and τ were determined to be 0.25 and 0.03, respectively. By changing the size of the sensitivity parameters, changes in the estimated effect size were examined in the instrumental model.

Sensitivity Analysis for Assumption Exclusion Restriction
If the IV (prescription preference) has a direct effect on EDSS change, then this assumption is violated. For example, a doctor who prefers the high potency drugs (IV = 1) simultaneously provides better accuracy and performance in treatment and therapeutic recommendations than physicians who prefer the low

Table 3. Estimating the Risk Differences Between High Potency and Low Potency for Different Values of The Sensitivity Parameters

Sensitivity Parameter Vector	δ	η	β	95%CI for Risk Difference
I	0	0	0.19	-0.10 -0.48
II	-0.001	0.10	0.01	-0.26 – 0.28
III	-0.010	0.10	0.01	-0.26 – 0.29
IV	-0.100	0.10	0.03	-0.24 – 0.30
V	0.001	0.10	0.01	-0.26 – 0.29
VI	0.100	0.10	0.00	-0.27 – 0.27
VII	0.300	0.20	0.09	-0.18 – 0.36
VIII	0.300	0.30	0.13	-0.14 – 0.40
IX	0.25	0.03	0.00	-0.27 – 0.27
X	0.50	0.03	-0.00	-0.27 – 0.26
XI	0.60	0.03	-0.01	-0.29 - 0.27
XII	0.70	0.10	-0.08	-0.36 – 0.20
XIII	0.80	0.20	-0.20	-0.48 – 0.09

δ: Effect of one SD increase in unmeasured confounder on mean of the EDSS change; η: Effect of the IV on unmeasured confounder; β: Risk difference estimate.

potency drugs (IV=0). The sensitivity parameter that investigates this violation from the ER assumption is called λ, and in fact, quantifies the effect modification of treatment efficacy in different layers of the IV.

For this purpose, if the outcome variable is defined as $Y_i - \lambda (1-Z_i)$, X_i is considered as the treatment variable, C_i as the measured confounder, and Z_i as the IV in the model,

$$Y_i^{z,x} = \alpha + \beta X_i + \Upsilon C_i + \lambda(1-Z_i) + \varepsilon_i \qquad E(\varepsilon_i \mid C_i, Z_i = 0)$$

Indeed, λ is the interaction coefficient between different treatment options and IV in the model. The sensitivity analysis showed the treatment effect estimation is robust in the instrumental model when λ value is changing from -0.20 to 0.10.

Discussion

IVA is recommended as a method for controlling the effect of unmeasured confounding in observational studies. Although the most important advantage of this approach is its lack of need for the "unmeasured confounders' assumption" to accurately estimate the treatment effect or adverse effects, using this advantage requires a thorough basic evaluation of the assumption and at the same time adopting a reasonable variance-bias tradeoff.[3,15,18]

For practicality, an example of a simple analytical framework was used in this study, and the IV was simply defined as a binary variable. Obviously, non-linear models, treatment effect heterogeneity or multiple IV methods need to consider more statistical and clinical concerns and the results of this study cannot simply be generalized to the community of patients.

The results of the primary analysis indicated that the improvement in disability in the high potency drug recipients was significantly higher than the low potency group by 0.27, and this difference increased to 0.4 after

adjusting for the known confounders. This finding revealed the success of the high potency treatment group in controlling the disability symptoms of patients after one year in comparison to low potency recipients. However, in addition to the various known risk factors, the decision to prescribe can be affected by many factors such as inherent and unknown prognostic features of patients or physician's character.

As previously stated, the first step in IVA is to ensure its proper use according to the study conditions. It seems that in this example, the role of unmeasured and unknown factors in the clinical outcomes of patients (the disability level) is completely clear, and the use of IVA can help to more accurately estimate the two treatment alternatives.[17,23,24] The evaluation of the first assumption (relevance assumption), that actually checks the strength of association between IV and intervention, indicated that this relationship was sufficient (using complier proportion, endogeneity test, and F statistics). This means that the chosen IV, like a randomization process, was able to allocate the patients to the IV groups. It should be noted that the confounding variables can be controlled for the sake of sufficient relationship of the IV with the treatment variable. Table 2 depicts the distribution of the measured confounding variables in various layers of the IV. Although an imbalance in the distribution is still seen in a variable such as recurrence, by looking at the general distribution of the confounders and at the homogeneity of this distribution, one can be sure of the other required assumption, i.e. the "effective random assignment."

The bias ratio is calculated to ensure that the IV affects the outcome only through its effect on the intervention pathway. This ratio shows lack of controlling the X confounder in the instrumental analysis in comparison to the OLS approach. The values of less than one indicate the optimality of the IV method compared to the usual OLS method.[8] As shown in Table 1, except for the treatment delay, this fraction is in favor of using

the IVA in the causal estimation. Therefore, one can be sure of achieving the IV independent of the unmeasured confounder (ERA) assumption in this analysis.

The main objective of this study was to compare the effectiveness of high potency interferon versus low potency interferon injection on patients' EDSS using IVA approach. Although in the estimation obtained from OLS in the linear regression model, the mean disability score decreased significantly by 0.4 in the high potency treatment group compared to the low potency group. The IVA showed that this improvement was 0.19 which was statistically non-significant (95% CI: -0.10-0.48). It seems that this reduction in the effect size can be attributed to the selection of specific patients for prescription of highly potent medication (bias by indication). In fact, unmeasured confounding due to lack of recognition of all confounders by researchers and residual confounding due to lack of complete control of unmeasured confounding variables can justify this variation in estimation Thus, this study did not finally conclude the superiority of the effect of the high potency treatment group against the low potency group (Table 3).

Obviously, the IVA can never control all the changes caused by the confounding variables due to lack of finding an ideal IV. For this reason, in addition to the basic assumption tests, it is always necessary to consider the results of the sensitivity analysis. The sensitivity analysis showed that in the case of a possible unmeasured confounder in the final model (assuming the largest confounding effect in the known variables like delay in diagnosis) over a wide range of changes in the confounding effect and also the presence of relationship between this variable and IV, the final effect size estimation is not statistically superior (Table 3). It was also found that in the case of different effect sizes in the layers of IV (heterogeneity in the range of -0.2 to 0.1), the IV model is still robust and the final conclusion is the lack of superiority of the high potency group.

Although the proposed example may have some shortcomings due to the study sample size, being limited to a certain number of neurologists, or choosing the EDSS variable in assessing the treatment efficacy, it has largely been able to provide the readers, in particular, the clinical researchers, with instrumental analysis application, reasons for use, necessary prerequisites, the effect size estimation, and sensitivity analysis.[15,25] Considering such a stepwise approach, it is suggested that the IVA can be used as the primary analysis when there are obvious impacts of unmeasured confounding on outcome. Otherwise, it can be used by researchers as an auxiliary analysis along with other methods of controlling confounding.

References
1. Velentgas P, Dreyer NA, Nourjah P, Smith SR, Torchia MM. Developing a Protocol for Observational Comparative Effectiveness Research: A User's Guide. Rockville, MD: Agency for Healthcare Research and Quality; 2013.
2. Bennett DA. An introduction to instrumental variables analysis: part 1. Neuroepidemiology. 2010;35(3):237-40. doi: 10.1159/000319455.
3. Ertefaie A, Small DS, Flory JH, Hennessy S. A tutorial on the use of instrumental variables in pharmacoepidemiology. Pharmacoepidemiol Drug Saf. 2017;26(4):357-67. doi: 10.1002/pds.4158.
4. Marko NF, Weil RJ. The role of observational investigations in comparative effectiveness research. Value Health. 2010;13(8):989-97. doi: 10.1111/j.1524-4733.2010.00786.x.
5. Meyer AM, Wheeler SB, Weinberger M, Chen RC, Carpenter WR. An overview of methods for comparative effectiveness research. Semin Radiat Oncol. 2014;24(1):5-13. doi: 10.1016/j.semradonc.2013.09.002.
6. Mansournia MA, Hernan MA, Greenland S. Matched designs and causal diagrams. Int J Epidemiol. 2013;42(3):860-9. doi: 10.1093/ije/dyt083.
7. Mansournia MA, Altman DG. Inverse probability weighting. BMJ. 2016;352:i189. doi: 10.1136/bmj.i189.
8. Mansournia MA, Etminan M, Danaei G, Kaufman JS, Collins G. Handling time varying confounding in observational research. BMJ. 2017;359:j4587. doi: 10.1136/bmj.j4587.
9. Mansournia MA, Danaei G, Forouzanfar MH, Mahmoodi M, Jamali M, Mansournia N, et al. Effect of physical activity on functional performance and knee pain in patients with osteoarthritis : analysis with marginal structural models. Epidemiology. 2012;23(4):631-40. doi: 10.1097/EDE.0b013e31824cc1c3.
10. Gharibzadeh S, Mohammad K, Rahimiforoushani A, Amouzegar A, Mansournia MA. Standardization as a Tool for Causal Inference in Medical Research. Arch Iran Med. 2016;19(9):666-70. doi: 0161909/aim.0011.
11. Shakiba M, Mansournia MA, Salari A, Soori H, Mansournia N, Kaufman JS. Accounting for Time-Varying Confounding in the Relationship Between Obesity and Coronary Heart Disease: Analysis With G-Estimation: The ARIC Study. Am J Epidemiol. 2018;187(6):1319-26. doi: 10.1093/aje/kwx360.
12. Almasi-Hashiani A, Nedjat S, Mansournia MA. Causal Methods for Observational Research: A Primer. Arch Iran Med. 2018;21(4):164-9.
13. Dekkers OM. On causation in therapeutic research: observational studies, randomised experiments and instrumental variable analysis. Prev Med. 2011;53(4-5):239-41. doi: 10.1016/j.ypmed.2011.08.003.

14. Hernan MA, Robins JM. Instruments for causal inference: an epidemiologist's dream? Epidemiology. 2006;17(4):360-72. doi: 10.1097/01.ede.0000222409.00878.37.

15. Baiocchi M, Cheng J, Small DS. Instrumental variable methods for causal inference. Stat Med. 2014;33(13):2297-340. doi: 10.1002/sim.6128.

16. Greenland S. An introduction to instrumental variables for epidemiologists. Int J Epidemiol. 2000;29(4):722-9.

17. Brookhart MA, Wang PS, Solomon DH, Schneeweiss S. Evaluating short-term drug effects using a physician-specific prescribing preference as an instrumental variable. Epidemiology. 2006;17(3):268-75. doi: 10.1097/01.ede.0000193606.58671.c5.

18. Brookhart MA, Rassen JA, Schneeweiss S. Instrumental variable methods in comparative safety and effectiveness research. Pharmacoepidemiol Drug Saf. 2010;19(6):537-54. doi: 10.1002/pds.1908.

19. Filippini G, Del Giovane C, Vacchi L, D'Amico R, Di Pietrantonj C, Beecher D, et al. Immunomodulators and immunosuppressants for multiple sclerosis: a network meta-analysis. Cochrane Database Syst Rev. 2013(6):Cd008933. doi: 10.1002/14651858.CD008933.pub2.

20. Kappos L, Freedman MS, Polman CH, Edan G, Hartung HP, Miller DH, et al. Effect of early versus delayed interferon beta-1b treatment on disability after a first clinical event suggestive of multiple sclerosis: a 3-year follow-up analysis of the BENEFIT study. Lancet. 2007;370(9585):389-97. doi: 10.1016/s0140-6736(07)61194-5.

21. Pittock SJ. Interferon beta in multiple sclerosis: how much BENEFIT? Lancet. 2007;370(9585):363-4. doi: 10.1016/s0140-6736(07)61170-2.

22. Torkildsen O, Myhr KM, Bo L. Disease-modifying treatments for multiple sclerosis - a review of approved medications. Eur J Neurol. 2016;23 Suppl 1:18-27. doi: 10.1111/ene.12883.

23. Boef AG, le Cessie S, Dekkers OM, Frey P, Kearney PM, Kerse N, et al. Physician's Prescribing Preference as an Instrumental Variable: Exploring Assumptions Using Survey Data. Epidemiology. 2016;27(2):276-83. doi: 10.1097/ede.0000000000000425.

24. Brookhart MA, Schneeweiss S. Preference-based instrumental variable methods for the estimation of treatment effects: assessing validity and interpreting results. Int J Biostat. 2007;3(1):Article 14.

25. Swanson SA, Hernan MA. Commentary: how to report instrumental variable analyses (suggestions welcome). Epidemiology. 2013;24(3):370-4. doi: 10.1097/EDE.0b013e31828d0590.