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Second-Order Interactions with the Treatment Groups in Controlled Clinical Trials

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Abstract Summary

The occurrence of significant second-order interactions for group characteristics was examined using real data in a randomized controlled trial (RCT). The interactions exist in all RCTs; they could be easily overlooked when using the simple randomization or stratification methods, but could become more obvious when minimization methods are used. Using real data from an RCT, the minimization method enabled balancing the distributions of the four selected stratified factors. Analyses for 3-way second-order interactions including 6 additional potential confounding variables (for a total of 10 variables) presented 8 significant second-order interactions with the treatment groups. Interaction effects need to be evaluated when treatment effects are examined to maximize the power of the treatment effects in any RCTs. A stepwise regression method with piecewise linear functions would be useful to select the significant variables with interaction effects affecting the treatment outcomes in RCTs. Additional ways to handle interaction effects in RCTs are presented in this paper.

Keywords

Second-Order Interactions; Minimization Random Allocation Method; RCTs

Introduction

The benefits and significance of evidence-based practice (EBP) have been widely acknowledged in the health care professions [1–3]. As research findings are used to advance the standards of care through EBP, reliable evidence from well-designed randomized clinical trials (RCTs) aimed at improved health outcomes is pivotal [2–3]. With the greater emphasis on the quality of RCTs, random allocation methods need to be examined more closely. In addition, as the scientific fields move forward, newly documented confounding variables identified for the population of interests on selected outcomes for the treatment effects need to

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be controlled by using more efficient random allocation methods. Thus, to advance the quality of RCT designs, the purpose of this paper is to present the real case examples with the confounding variables and their interactions with the treatment effects, using the computerized minimization method.

The random allocation of the research participants using the minimization method, and the occurrence of significant second-order interactions for group characteristics, were examined using real data in an RCT of high-risk mother-baby dyads. One hundred eighty eight dyads were randomly allocated into two groups using the minimization method, including four stratified variables selected based on prior studies in the population. Six additional variables (less significant, however could be additional confounders in the field) were used to examine the frequency of imbalance and magnitude of interactions in combinations. Three-way interactions (2^3 blocks) were computed by using multi-way contingency table analysis. The interactions exist in all RCTs; they could be easily overlooked when using the simple randomization or stratification methods, but could become more obvious when minimization methods are used. Interaction effects need to be evaluated when treatment effects are examined to maximize the power of the treatment effects in any RCTs.

Random Allocation

Random assignment of research participants into treatment conditions with blinding to the treatment is a significant quality indicator for the objectivity of the treatment effects in RCTs [4–7]. Random allocation is performed with the aim of balancing the distribution of confounding factors (variables) between two or more treatment groups. However, simple randomization cannot assure the balance of heterogeneous characteristics between and among groups, particularly when the sample size is fewer than 1,000 [8,9]. With the aid of computer programs, the minimization method not only enhances the objectivity of the random assignment; but also the feasibility of stratifying more than three binary confounding variables. This is the feasibility limit when using the stratified block randomization method [10]. The unbalanced covariates in an RCT would decrease the statistical power when comparing treatment effects. And, without balancing group confounders, erroneous conclusions could be derived [11,12]. Therefore, when additional confounding variables are identified for the outcomes, it is critical to balance and to control the newly documented confounding variables by using a more efficient random allocation method such as the minimization method.

Minimization Program

To enhance the quality and objectivity of the randomization, the computer-aided minimization method has been widely used to balance the confounding variables including subject characteristics between treatment groups in RCTs. Pocock and Simon's minimization method and Zelen's balancing method enable the balancing of each confounding factor (variable) between and among treatment subgroups over the entire duration of the trial [13–17]. The method was used increasingly in the 1990s due to the availability of computer programs [9]. The minimization method is more efficient than the simple random method and the stratified block randomization when measuring treatment effects, needing fewer subjects, by balancing confounding factors between and among treatment groups. Thus, a computer-aided minimization program can be used to not only balance the confounding variables, but to also enhance the objectivity, the efficiency, and the quality of randomization method for RCTs.

Three-Way or Second-Order Interactions in RCTs

The minimization method does not guarantee the balanced distribution of interactions of factors between the treatment groups, especially when additional significant confounding factors interacts with the treatment effects for the population of interests. However, the interaction

effects can be more clearly presented when using the minimization method, to suggest the needed controls in the analyses and the stratifications for future RCTs. A binary variable such as “race status” includes two marginal cells of “White” and “Non-white”, or “multiple birth status” includes two marginal cells of “singleton” and “twins”. When these two binary variables are associated, there are 2^2 or 4 cells (blocks). In binary 3-factor interactions, a third binary variable “sex of newborns”, with two marginal cells of “boys” and “girls”, would yield 2^3 or 8 cells, including interactions between all 3 pairs of variables.

In RCTs, if the interaction of factors is overlooked, it could yield a serious bias in estimating the treatment effects. For example, two medications are examined in an RCT, the new drug (D1) and the standard drug (D2). D1 has a much greater effect on male gender with positive complications than the D2. As the simple random assignment could not achieve balanced groups in the RCT, if the male patients in the D1 group accidentally have more complications at considerable greater effects, the treatment effect of the D1 group would be overestimated with complications. In this case, the distributions of “gender” and “the status of complications” might be completely balanced between D1 and D2 using the minimization method, considering the significant interaction effects for the treatments within the RCTs. In addition, the interactions of treatment effects could be compared using multiple regression models such as a logistic regression model and Cox’s proportional hazards model [18]. Although the need to address these interaction effects has been mentioned, and it would become more obvious when using the minimization method [10,18]; to-date, however, limited reports have presented the interaction effects using the minimization method.

Methods

Random allocation was performed using a web-based minimization program [19] [Figure 1] in a study involving high-risk mother-baby dyads. The RCT lasted 5 years at three tertiary care centers located in two metropolitan areas where both high-risk pregnant mothers and newborns could be cared for [20]. A total of 353 mothers agreed and gave their consent to participate in the study, with 388 newborns that included 33 pairs of twins and 1 set of triplets. There were 239 premature births. Mean gestational age at birth was 31 weeks, and birth weight was 1672 grams. Mother’s mean ages were 25 years. To qualify for the study, high-risk infants without severe congenital defects had to be intubated with an endotracheal tube; needed ventilatory support with respiratory failures, and also umbilical central line catheters to assess oxygenation and supply nutrients. The treatment group had advanced central-line monitoring of body’s oxygenation status, and the control group had the routine peripheral only monitoring. The courses of oxygen support and oxygenation complications for the hospital stay were the outcome variables of the study. Some cases that were not included in the trial because of emergency admissions with uncertain prognosis for high-risk of death, or being cared for by the clinicians who were unable to coordinate with the computerized allocation due to their lack of familiarity with the treatment allocations.

Random Allocation

Using the computerized minimization programs, we randomly allocated 188 cases (see Table 1). Random allocation in this RCT was done using Pocock and Simon’s minimization method [13] and Zelen’s method [17]. Appropriate human subjects approvals were obtained from institutional review boards. High-risk newborn babies were enrolled immediately after birth, with informed consents obtained from parents and appropriate guardians prior to delivery or right after delivery when they were accessible. Both a web-based program [19] and a DOS program [9] were used for joint 24-hour accessibility. Subjects were enrolled using the web-based program most of time; the DOS minimization program was used as a supplemental access.

The web-based program for the RCT was developed on the web server (UNIX workstation, SUN Microsystems SPARK Station 20), and the CGI script was written with PERL version 5.004 (Figure 1). The program was accessible to multiple users from clinical sites, and could be available through some carriers of wireless phone access to the website. The web-based program was designed with security features such as user authentication, firewall, and checking web contents to assure the confidentiality of the study subjects.

Four significant confounding variables were identified for the study outcomes, by reviewing the literature and by the results of an observational study [20–22]. These variables included race (white or other), delivery by cesarean (C-) section (yes or no), gestational age in weeks (≤ 28 weeks or > 28 weeks), and multiple-birth status (singleton or twins). To evaluate the interaction effects from these four variables and the treatment, a sample size of 160 subjects should be needed for this trial, for 4 binary factors in addition to the treatment group variable (with 5 subjects in each cell: $2 \times 2 \times 2 \times 2 \times 5 = 160$ total subjects). Additional six variables were less significantly associated to neonatal outcomes in the observational study or less feasible to assess during emergency admissions. Thus, they were not chosen in the trial because of the limited sample size, considering the interaction effects. These factors include gender (boy or girl) and birth weight (≤ 1.5 Kg or > 1.5 Kg) of newborns; and prenatal vaginal bleeding (yes or no), prematurely ruptured membrane (yes or no), prenatal antibiotic treatments (yes or no), and prenatal steroid treatments (yes or no) of mothers.

Statistical Analysis

Statistical software [Statistical Package for Social Sciences (SPSS) and the Statistical Analysis System (SAS)] was used for the analysis. Three-way interactions (2^3 blocks) were computed by using multi-way contingency table analysis in the SAS program. To present the interaction effects, all 10 variables were analyzed for their potential interactions. Additional bivariate chi-square or Fisher's exact tests (2^2 blocks) were performed within each treatment group to examine the post-hoc analyses for the variables that showed significant three-way interactions.

Results

Table 1 presents the balance of the factors and cell distribution at two stages of trial with respective sample sizes (A = at an early stage of the trial, B = at the completion stage). The values represent the extent of imbalances for the factors (Table 1 A & B). Respective imbalances were 22% of total subject dyads (12 for $n = 55$) at the early stage and only 2% of total dyads (4 for $n = 188$) at the completion stage of the trial. Thus, the imbalances for these strata-factors got smaller as the sample size increased.

Table 2 includes six additional variables that were not selected in the trial because they were less significantly associated with the health outcomes than the variables selected. The imbalance from these six additional variables was 40% of the total subject dyads (76 for $n = 188$). Among these additional variables, only prenatal steroid use of the mothers was significantly different between the treatment groups ($P < 0.05$).

Table 3 presents 42 P-values for three-way interactions using multi-way contingency table analysis, including the treatment groups and two other variables for the second-order interactions. Three-way interaction could not be computed in three situations where there were 0 frequencies (or lack of responses) in the following three combinations: multiple-birth and race (and the treatment groups), multiple-birth and prenatal vaginal bleeding (and the treatment groups), and gestational age and birth weight (and the treatment groups). Of the 42 possible combinations, 8 combinations denoted by asterisk (*) had significant interactions ($P < 0.05$, the distributions of the corresponding variables were significantly different between the

treatment groups). Particularly, a significant interaction was detected in the combination of two stratified variables, multiple-birth and C-section (and the treatment groups).

For the significant three-way interactions, additional bivariate Fisher's exact tests were performed within each treatment subgroup for the post-hoc analyses of significance, to clarify the interaction effects. In Table 4, the variables that had significant interactions with multiple-birth status within the treatment groups were presented. In Table 5, variables that had significant interactions with prenatal vaginal bleeding status were presented. And, in Table 6, variables that had significant interactions with newborn gender were presented. To summarize the significant interactions presented in Table 4, more twins (as compared to singletons) in the treatment group (compared to the control group) had C-section; but more twins (rather than singletons) in the control group (compared to the treatment group) had prenatal premature rupture of membranes and antibiotics use.

The following pairs presented no responses in the factor cells: multiple-birth with race and multiple-birth with prenatal vaginal bleeding. These lack of responses were implicit from the uneven distributions ($< 20\%$ and $> 80\%$ responses) presented in Table 1 and Table 2. To summarize further the findings from Table 5, more newborns whose mothers had prenatal vaginal bleeding (as compared to those who did not) in the control group (compared to the treatment group) were bigger babies (> 1.5 Kg). In addition, more babies whose mothers had prenatal vaginal bleeding (as compared to those who did not) in the treatment group (compared to the control group) had mothers with prenatal premature rupture of membrane and antibiotics use. Furthermore, as presented in Table 6, more boys (than girls) in the treatment group (compared to the control group) were younger babies (≤ 28 weeks gestation) and had mothers with prenatal premature rupture of membrane. The differences of the distributions between groups had occurred by chance in this trial. These interactions could not have been avoided in any RCTs, but became clearer using the minimization method.

Discussion

Efficient allocation programs for the treatment groups are needed for future RCTs to ensure the quality of RCTs and efficient designs. The minimization method is an efficient computerized random allocation method. It can be used to better control and balance potential confounding variables for the assessment of treatment effects. The occurrence of interactions among variables became clearer using the minimization method. Examination of interaction effects among variables could be useful in RCTs as interactions among variables could yield significant differences of the subject characteristics for the treatment effects.

When comparing treatment effects using univariate analysis (using logrank test for survival times and Fisher's exact test for binary response for example), different distributions may not affect the comparisons of treatment effects. However, galloping alpha and multiple Bonferroni corrections would decrease the power of the treatment effects. In addition, when multivariate analysis such as Cox's proportional hazards model and multiple logistic regression analysis is used to evaluate the treatment effects, the negligence of second-order interaction terms can lead to a serious bias in the evaluation of treatment effects [18]. Therefore, it is important to investigate the frequency and the magnitude of interactions of confounding variables in RCTs. Thus, the findings in this paper could provide valuable information to construct multivariate model for selecting significant strata to evaluate the treatment effects.

Second-order interaction terms have not always been taken into consideration when constructing multivariate models. Our study suggests that the second-order interactions could have significantly different distributions, and thus need to be considered for the models of analyses. For a practical statistical analysis, if there are ten confounding variables to be

considered, the number of interaction terms generated by the combinations of three variables, is 45. This is a significant number of combinations to consider for any RCTs, but needs attention for potential interaction terms. As the scientific fields move forward with more reliable results from RCTs, there is a greater need for networking within similar fields to be familiar with related confounding variables and second-order interactions for the population of interests when examining the treatment effects.

In a regression analysis to evaluate treatment effects in clinical trials, researchers often use a forward or backward stepwise regression method to select the significant confounding variables in an RCT. Concrete examples for constructing a regression model including second-order interaction terms have been referred to in some references [18]. When the variables x_1 and x_2 are continuous or binary, the second-order interaction term can simply be created by the multiplication of x_1 and x_2 . However, in clinical trials, some variables are often ordered, which have more than three categories. For example, a lymph node metastasis in cancer clinical trials has four categories, they are, 0:none, 1:slight, 2:intermediate, 3:severe. With the variables having four categories, the second-order interaction terms can be created by the products of piecewise linear functions $\langle x_1 - i \rangle$ and $\langle x_2 - j \rangle$, where $\langle x_1 - i \rangle = \{(x_1 - i) + |x_1 - i|\} / 2$; the suffix i and j indicate the i -th category of x_1 and the j -th category of x_2 , respectively [24].

Future Plans

A minimization method has demonstrated its efficiency for quality random allocation method in RCTs, as the best method to balance the distribution of confounding factors between and among the treatment groups. The occurrence of significant interactions cannot be avoided in any RCTs, but will become clearer when using a minimization method. Therefore, interactive effects of the confounding factors for the population of interest must be considered when evaluating the treatment effects in RCTs. Once the interaction effects of the confounding variables have been identified within the treatment groups, future RCTs using the minimization method could design the trials to include the interactive cells between the two strata as additional factors for outcome evaluations. A stepwise regression method with piecewise linear functions would be useful to select the significant interactions affecting the outcomes in RCTs [18,24]. In this era of international collaboration, a web-based minimization program enables the balancing of the significant confounding variables, for a more efficient design and better quality RCTs. As wireless telephone technology has become conveniently available in recent years to access websites, a web-based program will be an excellent resource for multi-site studies to balance cases more efficiently in experiments.

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Figure 1.

Web access to allocate subjects using minimization method, supported by Kyushu University Hospital, Japan, with login security.

Table 1

Balance of cases per groups for four minimization variables with different sample sizes:

A. n = 55					
Variables	Groups	Control	Experimental	Subtotal	Imbalance
Race					
	Other	16	16	32	0
	White	12	11	23	1
C-section					
	No	9	7	16	2
	Yes	19	20	39	1
Gestation Age, weeks					
	< 28	6	5	11	1
	> 28	22	22	44	0
Multiple Birth Status					
	Single	26	22	48	4
	Twins	2	5	7	3
	Total (% Total)	28 (51%)	27 (49%)	55 (100%)	12 (22%)
B. n = 188					
Race					
	Other	68	67	135	1
	White	26	27	53	1
C-section					
	No	36	36	72	0
	Yes	58	58	116	0
Gestation Age, weeks					
	< 28	30	30	60	0
	> 28	64	64	128	0
Multiple Birth Status					
	Single	80	79	159	1
	Twins	14	15	29	1
	Total (% Total)	94 (50%)	94 (50%)	188 (100%)	4 (2%)

Table 2

Balance of cases per groups on additional five related variables as comparison (n = 188).

Variables	Groups	Control	Experimental	Subtotal	Imbalance
Gender					
	Boy	54	49	103	5
	Girl	40	45	85	5
Birth Weight, Kg					
	< 1.5	46	54	100	8
	> 1.5	48	40	88	8
Vaginal Bleeding					
	No	82	83	165	1
	Yes	12	11	23	1
Ruptured Membrane					
	No	66	63	129	3
	Yes	28	31	59	3
Prenatal Antibiotics					
	No	45	38	83	7
	Yes	49	56	105	7
Prenatal Steroid*					
	No	65	51	116*	14
	Yes	29	43	72*	14
	Total (% Total)	94 (50%)	94 (50%)	188 (100%)	76 (40%)

* P < .05

Table 3

Significant interactions (* **P values listed**) at 3 factorial levels ($2 \times 2 \times 2 = 8$ cells each) for listed paired variables and experimental groups.

	C-section	Race	Multiple	Weight	Age	Bleed	Rupture	Antib	Steroid
Race	0.964								
Multiple	0.008*	NA							
Weight	0.093	0.922	0.345						
Age	0.940	0.455	0.365	NA					
Bleed	0.964	0.617	NA	0.001*	0.082				
Rupture	0.746	0.988	0.002*	0.194	0.691	0.022*			
Antibiotics	0.611	0.629	0.012*	0.810	0.148	0.011*	0.404		
Steroid	0.869	0.902	0.591	0.849	0.286	0.518	0.372	0.772	
Gender	0.340	0.881	0.614	0.273	0.008*	0.366	0.023*	0.306	0.605

NA: 3-way interactions could not be calculated with lack of response in 1 of 8 cells

Table 4

Significant interactions for 3 factors and 2 factors within each group for multiple birth Status.

Significant interactions for 3 factors and 2 factors within each group for multiple birth status:										
		Control			P (2×2)	Experimental			P (2×2)	P (2×2×2)
		Multiple status				Multiple status				
Variables	Groups	Single	Twin	Subtotal		Single	Twin	Subtotal		
C-Section					0.829				0.03 [*]	0.008 ^{**}
	No	31	5	36		34	2	36		
	Yes	49	9	58		45	13	58		
Race					0.063				0.007 ^{**}	NA
	Other	55	13	68		52	15	67		
	White	25	1	26		27	0	27		
Bleeding					0.121				0.124	NA
	No	68	14	82		68	15	83		
	Yes	12	0	12		11	0	11		
Rupture					0.002 ^{**}				0.078	0.002 ^{**}
	No	61	5	66		50	13	63		
	Yes	19	9	28		29	2	31		
Antibiotics					0.117				0.092	0.012 [*]
	No	41	4	45		29	9	38		
	Yes	39	10	49		50	6	56		
	N Total	80	14	94		79	15	94		188

* p < .05,

** P < .01;

NA: Not computable for lack of (or 0) responses in one of the cells.

Table 5

Significant interactions for 3 factors and 2 factors within groups for vaginal bleeding status.

Significant interactions for 3 factors and 2 factors within groups for vaginal bleeding status.										
		Control			P (2×2)	Experimental			P (2×2)	P (2×2×2)
		Bleeding				Bleeding				
Variables	Groups	No	Yes	Subtotal		No	Yes	Subtotal		
Baby weight, Kg					0.076				0.082	0.001***
	<1.5	43	3	46		45	9	54		
	> 1.5	39	9	48		38	2	40		
Rupture					0.287				0.105	0.022*
	No	56	10	66		58	5	63		
	Yes	26	2	28		25	6	31		
Antibiotics					0.874				0.024*	0.011*
	No	39	6	45		37	1	38		
	Yes	43	6	49		46	10	56		
	N Total	82	12	94		83	11	94		188

* P < .05,

p < .001.

Table 6

Significant interactions for 3 factors and 2 factors within groups for gender.

		Control			p (2×2)	Experimental			p (2×2)	p (2×2×2)	
		Gender				Gender					p (2×2)
Variables	Groups	Girl	Boy	Subtotal		Girl	Boy	Subtotal			
Age, weeks					0.148				0.296	0.008 ^{**}	
	< 28	16	14	30		12	18	30			
	> 28	24	40	64		33	31	64			
Rupture					0.159				0.212	0.023 [*]	
	No	25	41	66		33	30	63			
	Yes	15	13	28		12	19	31			
	N Total	40	54	94		45	49	94		188	

* P < .05,

** P < .01.