

On the mixed-model analysis of covariance in cluster-randomized trials

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Abstract

In the analyses of cluster-randomized trials, a standard approach for covariate adjustment and handling within-cluster correlations is the mixed-model analysis of covariance (ANCOVA). The mixed-model ANCOVA makes stringent assumptions, including normality, linearity, and a compound symmetric correlation structure, which may be challenging to verify and may not hold in practice. When mixed-model ANCOVA assumptions are violated, the validity and efficiency of the model-based inference for the average treatment effect are currently unclear. In this article, we prove that the mixed-model ANCOVA estimator for the average treatment effect is consistent and asymptotically normal under arbitrary misspecification of its working model. Under equal randomization, we further show that the model-based variance estimator for the mixed-model ANCOVA estimator remains consistent, clarifying that the confidence interval given by standard software is asymptotically valid even under model misspecification. Beyond robustness, we also provide a caveat that covariate adjustment via mixed-model ANCOVA may lead to precision loss compared to no adjustment when the covariance structure is misspecified, and describe when a cluster-level ANCOVA becomes more efficient. These results hold under both simple and stratified randomization, and are further illustrated via simulations as well as analyses of three cluster-randomized trials.

Keywords: Average treatment effect; Covariate adjustment; Group-randomized trials; Model-based variance; Linear mixed model; Stratified randomization.

1 Introduction

Cluster-randomized trials (CRTs) are increasingly used to study interventions and inform decision-making in real-world settings. Distinct from individually-randomized trials, CRTs randomize groups of individuals, such as a hospital, classroom, or village, to treatment conditions. Cluster-level treatment assignment is carried out when individual-level randomization is infeasible or when there are concerns on treatment contamination under individual randomization (Murray et al., 1998; Donner and Klar, 2000). A distinguishing feature of CRTs is that observations collected within the same cluster tend to be correlated, which is an important aspect that must be reflected during both the design and analysis stages.

The linear mixed model is by far the most popular regression approach to account for within-cluster correlation when estimating the average treatment effect in CRTs. For example, the systematic review by Fiero et al. (2016) suggested that 52% of the published CRTs between August 2013 and July 2014 used mixed models in their primary analyses. The mixed-model analysis of covariance (mixed-model ANCOVA, following the terminology in Section 4 of Murray et al., 1998) has been shown in simulation studies to improve the study power when the mixed model is correctly specified (Raudenbush, 1997; Li et al., 2016), while other simulation studies (Zhang and Davidian, 2001; Litière et al., 2007, 2008; McCulloch and Neuhaus, 2011) indicated that non-normality can result in efficiency loss of linear mixed models. No analytical insights, however, were given on whether the power gain of mixed-model ANCOVA compared to the unadjusted analysis persists with misspecified models.

In addition to precision, the validity of misspecified mixed-model ANCOVA is not clear, either. When the random-effects distribution and/or the residual error distribution is misspecified, Murray et al. (2006) showed by simulations that the mixed-model ANCOVA maintained a valid type I error rate under equal randomization of clusters, but provided no theoretical justifications. In the more broad research area of generalized linear mixed models, robustness to misspecification of random effect distribution has been extensively studied (McCulloch and Neuhaus, 2011; Neuhaus et al., 2013; Jiang, 2017; Drikvandi et al., 2017),

given the assumption that the first moment of the generalized linear mixed model is correctly specified conditioning on random effects. This assumption, however, excludes misspecification of the functional form of baseline covariates and the correlation structure among individuals, both of which can be concerned in practice. As a result, the validity of mixed-model ANCOVA for estimating the average treatment effect in CRTs remains unclear under *arbitrary* model misspecification, including linearity on covariates, normality of the outcomes, inter-subject correlation structure that depends on covariates, and etc.

In this article, we study the robustness and efficiency properties of the mixed-model ANCOVA estimator for the average treatment effect in CRTs. The mixed-model ANCOVA extends the “ANCOVA I” in [Yang and Tsiatis \(2001\)](#) and involves regressing the individual-level outcomes on fixed effects for the intercept, treatment, and covariates, as well as a normally-distributed cluster-level random intercept. We label a model-based estimator “robust” if it is consistent and asymptotically normal under arbitrary misspecification of its working model. When the model assumptions are challenging to verify or unlikely to hold, such as the linear mixed model, robustness is a desired property for valid statistical inference. Under certain regularity conditions, we first prove that the mixed-model ANCOVA estimator for the average treatment effect across clusters is robust to arbitrary model misspecification. We further show that, under equal randomization, the model-based variance estimator under mixed-model ANCOVA remains consistent under arbitrary model misspecification. These analytical insights extend the results developed in [Wang et al. \(2019\)](#) from individually randomized trials to CRTs, thereby providing a new theoretical basis to justify the mixed-model ANCOVA in the analysis of CRTs.

In addition to the robustness property, we also assess the efficiency of the mixed-model ANCOVA estimator and contribute a surprising result that covariate adjustment by the mixed-model ANCOVA may *not* always increase precision of the average treatment effect estimator, even under equal randomization. This result is in sharp contrast to findings for individually-randomized trials where, asymptotically, covariate adjustment by ANCOVA leads to no loss in precision ([Yang and Tsiatis, 2001](#); [Tsiatis et al., 2008](#)). Somewhat counter-

intuitively, we further demonstrate that, assuming equal randomization and constant cluster sizes, when covariate adjustment by the mixed-model ANCOVA estimator leads to precision gain, such a precision improvement does not exceed that from covariate adjustment by a cluster-level ANCOVA model, namely, ANCOVA on cluster-level means aggregated from individual-level outcomes. These new insights are further demonstrated by a simulation study and analysis of three real CRTs.

The present article builds on existing work on robust casual inference for CRTs. The majority of prior work on the estimation and inference of the average treatment effect in CRTs is embedded in the randomization-inference framework, where the randomness of potential outcomes is dictated by the cluster-level treatment assignment. Here we focus on the super-population framework, which is more commonly invoked in the analysis of randomized clinical trials. A comparison of the randomization-inference and super-population frameworks can be found in [Robins \(2002\)](#). [Small et al. \(2008\)](#) first developed the randomization-inference estimator for the average treatment effect assuming a constant effect size across all units. [Imai et al. \(2009\)](#) and [Middleton and Aronow \(2015\)](#) performed cluster-level analyses for the average treatment effect in CRTs, where estimators were constructed using cluster totals or averages. By collapsing observations at the cluster level, their estimators avoid dealing with the intracluster correlation coefficient among individual-level observations. More recently, [Park and Kang \(2021\)](#), [Schochet et al. \(2021\)](#) and [Su and Ding \(2021\)](#) considered individual-level linear regression methods under the randomization-inference framework, with slightly different emphases on network causal effects under non-compliance, blocked designs, and efficiency improvement, respectively. To the best of our knowledge, no prior work have investigated the robustness and asymptotic efficiency of the average treatment effect estimator based on mixed-model ANCOVA, which nevertheless is more commonly used for analyzing CRTs.

Unlike individually-randomized trials where the data for each individual are assumed to be independent such that the central limit theorem can be directly applied, CRTs yield correlated data, leading to potential challenges in proving asymptotic results. To deal with this

additional complexity, we contribute a causal framework that converts a CRT into a repeat-measure unit-randomized trial; cluster size variation is also handled by conceptualizing it as a missing data problem. Under our framework, we were able to derive the asymptotic distribution of the mixed-model ANCOVA estimator for the average treatment effect by invoking the appropriate semiparametric theory (van der Vaart, 1998) and then used matrix theory to arrive at our final results which account for the correlated nature of the outcomes within clusters.

The remainder of the article is organized as follows. In Section 2, we describe our super-population framework and present the structural assumptions for identifying the average treatment effect in CRTs. Section 3 gives our main results, including the asymptotic distribution of the mixed-model ANCOVA estimator, a formal characterization of precision gain from covariate adjustment, as well as a generalization of these asymptotic results to stratified randomization. In Section 4, we demonstrate our theoretical results via simulation studies. In Section 5, we re-analyze three real-world CRTs to comprehensively illustrate the performance of the unadjusted and mixed-model ANCOVA estimators. Section 6 concludes.

2 Notation, Assumptions and Mixed-Model ANCOVA in CRTs

2.1 General Setup

We consider a CRT with m clusters. Each cluster i , $i = 1, \dots, m$, contains at least n individuals, whereas only N_i individuals are recruited and observed in the study, leading to potentially varying observed cluster sizes. We assume that $2 \leq N_i \leq n$, namely, the number of recruited individuals in each cluster is upper bounded by n but is at least 2. For example, when clusters represent hospitals and each hospital can have more than $n = 200$ patients that potentially satisfy the inclusion criteria, the study may only enroll $N_i \in [50, 100]$ patients from each hospital. In practice, n can represent the number of individuals in the source

population of interest and can be substantially larger than N_i .

In a CRT, the treatment is assigned at the cluster level instead of the individual level; individuals in the same cluster therefore are assigned the same treatment. For each cluster i , we define A_i as the treatment indicator ($A_i = 1$ if treated and 0 otherwise). For each individual $j = 1, \dots, n$ in cluster i , we define Y_{ij} as the continuous outcome and $\mathbf{X}_{ij} \in \mathbb{R}^p$ as a vector of baseline covariates. Here \mathbf{X}_{ij} can contain both individual-specific information (X_{ij} varies across different individual j in the same cluster i) and cluster-level information (X_{ij} is constant across individual j in the same cluster i). We pursue the potential outcome framework and assume consistency such that for each individual j in cluster i ,

$$Y_{ij} = A_i Y_{ij}(1) + (1 - A_i) Y_{ij}(0),$$

where $Y_{ij}(a)$ is the potential outcome of individual j in cluster i if cluster i were assigned treatment a for $a \in \{0, 1\}$. We note that the counterfactual model is defined based on a hypothetical *cluster-* rather than *individual-* level intervention. We further define the complete (but not fully observed) data vector for individual j in cluster i as $\mathbf{W}_{ij} = (Y_{ij}(1), Y_{ij}(0), \mathbf{X}_{ij})$, and the complete data vector for cluster i as $\mathbf{W}_i = (\mathbf{W}_{i1}, \dots, \mathbf{W}_{in})$. To proceed, we make the following structural assumptions on $(\mathbf{W}_1, \dots, \mathbf{W}_m)$ and the assignment vector (A_1, \dots, A_m) .

Assumption 1. (Super-Population Sampling and Cluster Randomization)

- (a) $\{\mathbf{W}_i, i = 1, \dots, m\}$ are independent and identically-distributed samples from the joint distribution $\mathcal{P}^{(\mathbf{W})}$ on the random vector $\mathbf{W} = (\mathbf{W}_{\bullet,1}, \dots, \mathbf{W}_{\bullet,n})$.
- (b) Within $\mathcal{P}^{(\mathbf{W})}$, each $\mathbf{W}_{\bullet,j}$ ($j = 1, \dots, n$) follows a common distribution \mathcal{P} on $(Y(1), Y(0), \mathbf{X})$. In other words, $\mathbf{W}_{\bullet,1}, \dots, \mathbf{W}_{\bullet,n}$ are marginally identically distributed.
- (c) The cluster-level treatment assignment, $\{A_i, i = 1, \dots, m\}$ are independent, identically distributed samples from a Bernoulli distribution $\mathcal{P}^{(A)}$ on A with marginal probability $P(A = 1) = \pi \in (0, 1)$. Furthermore, (A_1, \dots, A_m) is independent of $(\mathbf{W}_1, \dots, \mathbf{W}_m)$.

Assumption 1(a) implies that the data vector for each cluster is a random sample from a common distribution $\mathcal{P}^{(\mathbf{W})}$. Of note, this assumption is necessary to introduce the super-

population framework, but does not assume homogeneous treatment effects across clusters, because the conditional distribution of \mathbf{W}_i given observed $(\mathbf{X}_{i1}, \dots, \mathbf{X}_{in})$ is still allowed to vary across clusters. Assumption 1(b) requires that, for each individual in the same cluster i , their complete data vectors follow the same marginal distribution, whereas they can be marginally correlated. Analogously, the conditional correlation structure among individuals of the same cluster can also vary across clusters. Finally, Assumption 1(c) is the treatment randomization assumption that holds under the cluster randomization design.

While Assumption 1 elucidates conditions for the complete data vector, we require an additional assumption on the observed data vector. For $i = 1, \dots, m$ and $j = 1, \dots, n$, we define a latent random variable M_{ij} as an indicator of whether individual j from cluster i is enrolled in the study ($M_{ij} = 1$) or not ($M_{ij} = 0$). We further define $\mathcal{O}_i = \{j : M_{ij} = 1\}$, the index set of enrolled individuals, which contains $N_i = \sum_{j=1}^n M_{ij}$ elements. The observed data for cluster i is therefore $\{(Y_{ij}, A_i, \mathbf{X}_{ij}) : j \in \mathcal{O}_i\}$. Assumption 2 underlies the connection between the complete data and the observed data.

Assumption 2. (Non-informative Enrollment)

Denote $\mathbf{M}_i = (M_{i1}, \dots, M_{in})$ as the collection of latent enrollment indicators. $\{\mathbf{M}_i, i = 1, \dots, m\}$ are independent, identically distributed samples from a common distribution $\mathcal{P}^{(\mathbf{M})}$ on \mathbf{M} . Furthermore, $(\mathbf{M}_1, \dots, \mathbf{M}_m)$ is independent of $(\mathbf{W}_1, \dots, \mathbf{W}_m)$ and (A_1, \dots, A_m) .

Assumption 2 implies that, within each cluster, the enrollment of individuals, as well as the cluster size N_i , is random and independent of the remaining data information, including the potential outcomes, treatment, and baseline covariates. In addition, Assumption 2 allows for unequal cluster sizes, whose randomness can be attributed to logistical and operational uncertainties across clusters but is otherwise unrelated to the potential outcomes. A similar non-informative cluster size assumption has been routinely invoked in the CRT literature, especially for purposes of sample size calculation (Eldridge et al., 2006). In our setup, Assumption 2 is required for unbiased estimation of the average treatment effect across clusters (defined as Δ_2 in Section 2.2) using mixed-model ANCOVA, where clusters with more individuals enrolled contribute more information to the average treatment effect

estimator. Finally, Assumption 2 will be violated when the cluster-specific average treatment effect depends on the cluster size (Seaman et al., 2014), or when informative enrollment of individuals by treatment conditions leads to selection bias (Li et al., 2021). We will return to a discussion of these more challenging scenarios in Section 6.

2.2 Causal Estimands under the Super-Population Framework

Our goal is to estimate the average treatment effect, defined as

$$\Delta^* = E\{Y_{ij}(1)\} - E\{Y_{ij}(0)\},$$

which compares the expected individual-level potential outcomes if a cluster were assigned treatment versus control. Given Assumptions 1 and 2, the estimand can also be written as

$$\Delta^* = E\{\bar{Y}_i(1)\} - E\{\bar{Y}_i(0)\},$$

where $\bar{Y}_i(a) = n^{-1} \sum_{j=1}^n Y_{ij}(a)$ is the averaged potential outcomes for individuals in cluster i if the cluster i were assigned treatment a . Hence, the estimand Δ^* is also the average treatment effect of cluster averages across clusters.

Under the randomization-inference framework, Su and Ding (2021) discussed two different estimands, which are the average treatment effect across enrolled individuals, $\Delta_1 = \sum_{i=1}^m N_i \tau_i / \sum_{i=1}^m N_i$, and the average treatment effect across clusters, $\Delta_2 = \sum_{i=1}^m \tau_i / m$, where $\tau_i = N_i^{-1} \sum_{j \in \mathcal{O}_j} \{Y_{ij}(1) - Y_{ij}(0)\}$ is the average of contrast in potential outcomes among the enrolled individuals in cluster i , and $Y_{ij}(a)$ and N_i are treated as fixed quantities. These two estimands differ on how τ_i is weighted: Δ_1 weights τ_i by the number of individuals enrolled in cluster i ; Δ_2 assigns equal weight across clusters regardless of their sample sizes. Given our Assumptions 1 and 2 under the super-population framework, we can show that $\Delta^* = E[\Delta_1] = E[\Delta_2]$, which unifies Δ_1 and Δ_2 by marginalizing over the randomness in $Y_{ij}(a)$ and N_i ; therefore, our estimand Δ^* can be interpreted as the average treatment effect among the population of interest or the average treatment effect across clusters.

2.3 The Mixed-Model ANCOVA Estimator

We focus on a continuous outcome, for which the mixed-model ANCOVA is given by, for $i = 1, \dots, m$ and $j = 1, \dots, n$,

$$Y_{ij} = \beta_0 + \beta_A A_i + \boldsymbol{\beta}_{\mathbf{X}}^\top \mathbf{X}_{ij} + \delta_i + \epsilon_{ij}, \quad (1)$$

where $\delta_i \sim N(0, \tau^2)$ is the random effect for cluster i , $\epsilon_{ij} \sim N(0, \sigma^2)$ is the residual error for individual j in cluster i , and $(\beta_0, \beta_A, \boldsymbol{\beta}_{\mathbf{X}}, \sigma^2, \tau^2)$ represent unknown parameters. Typically in CRTs, the mixed-model ANCOVA assumes that elements of $(\delta_1, \dots, \delta_m, \epsilon_{11}, \dots, \epsilon_{m,n})$ are mutually independent and are further independent of the treatment assignment (A_1, \dots, A_m) and all covariates $(\mathbf{X}_{11}, \dots, \mathbf{X}_{m,n})$. Under this model, the proportion of total variance of Y_{ij} that is attributable to the between-group variation, $\tau^2/(\tau^2 + \sigma^2)$, is typically referred to as the intraclass correlation coefficient (Murray et al., 1998).

We consider maximum likelihood estimators of $(\beta_0, \beta_A, \boldsymbol{\beta}_{\mathbf{X}}, \sigma^2, \tau^2)$ based on the observed data $\{(Y_{ij}, A_i, \mathbf{X}_{ij}) : j \in \mathcal{O}_i, i = 1, \dots, m\}$, and denote them by $(\hat{\beta}_0, \hat{\beta}_A, \hat{\boldsymbol{\beta}}_{\mathbf{X}}, \hat{\sigma}^2, \hat{\tau}^2)$. We refer to Jiang (2017) for full technical details on maximum likelihood estimation of linear mixed models, based on which we derive our key results. In CRT applications, the average treatment effect parameter, Δ^* , is often estimated by $\hat{\beta}_A$, which we denote as $\hat{\Delta}$ and refer to as the mixed-model ANCOVA estimator from hereon in. We also denote the model-based variance estimator for $\hat{\Delta}$ as $\widehat{Var}(\hat{\Delta})$, which is given by the second-row, second-column entry of the inverse

$$\left\{ \sum_{i=1}^m \mathbf{Q}_i^o \top \hat{\boldsymbol{\Sigma}}_i^{-1} \mathbf{Q}_i^o \right\}^{-1},$$

where $\mathbf{Q}_i^o = (\mathbf{1}_{N_i}, A_i \mathbf{1}_{N_i}, \mathbf{X}_i^o)$ is the $N_i \times (p + 2)$ design matrix for cluster i and $\hat{\boldsymbol{\Sigma}}_i = m/(m - p - 2)(\hat{\sigma}^2 \mathbf{I}_{N_i} + \hat{\tau}^2 \mathbf{1}_{N_i} \mathbf{1}_{N_i}^\top)$ is the estimated covariance structure for cluster i (with adjustment for the degrees of freedom), where $\mathbf{1}_{N_i}$ is a N_i -dimensional column vector of ones, \mathbf{I}_{N_i} is the $N_i \times N_i$ identity matrix, and $\mathbf{X}_i^o = (\mathbf{X}_{i,j_1}, \dots, \mathbf{X}_{i,j_{N_i}})^\top$ with (j_1, \dots, j_{N_i}) being the distinct elements of \mathcal{O}_i .

For deriving our main theoretical results for the mixed-model ANCOVA estimator, we assume additional regularity conditions on the derivative of the log-likelihood function corresponding to the mixed-model ANCOVA (1), which are given in the Supplementary Material. These regularity conditions are essentially moment and continuity conditions required for proving the asymptotic normality of $\widehat{\Delta}$ and are similar to conditions invoked in Theorem 5.41 of [van der Vaart \(1998\)](#).

3 Main Theoretical Results

Our main results below hold under arbitrary model misspecification. The first result, in Section 3.1, is the robustness of the mixed-model ANCOVA point and variance estimator. Second, in Section 3.2, we clarify the precision gain by covariate adjustment via mixed-model ANCOVA, and discuss possible efficiency improvement by instead analyzing cluster-specific means. In Section 3.3, we extend the above results to stratified cluster randomization, which is standard practice for design-based control of covariates in CRTs with a relatively small number of clusters.

3.1 Robustness of the Mixed-Model ANCOVA Estimator

Theorem 1. *(a) Under Assumptions 1, 2 and additional regularity conditions outlined in the Supplementary Material, the mixed-model ANCOVA estimator $\widehat{\Delta}$ for Δ^* is consistent, i.e., $\widehat{\Delta}$ converges in probability to Δ^* as $m \rightarrow \infty$, and is asymptotically normal, i.e., $\sqrt{n}(\widehat{\Delta} - \Delta^*)$ converges weakly to a normal distribution $N(0, v)$, under arbitrary misspecification of its working model. The explicit form of v is given in the Supplementary Material. (b) Furthermore, under equal randomization with $\pi = 0.5$, $m\widehat{Var}(\widehat{\Delta})$ converges in probability to the true asymptotic variance v , and therefore the model-based variance estimator $\widehat{Var}(\widehat{\Delta})$ remains valid.*

Theorem 1 provides a formal statement on the robustness of the mixed-model ANCOVA estimator for the average treatment effect in CRTs under arbitrary model misspecification.

That is to say, even when the conditional mean structure, covariance structure of the random effects, and/or other aspects of residual error distribution are incorrect, the bias of the resulting estimator $\widehat{\Delta}$ vanishes with an increasing number of clusters. Theorem 1(a) extends existing results developed for the simple ANCOVA estimator under individually randomized trials (Yang and Tsiatis, 2001; Lin, 2013) to CRTs with correlated outcomes. It also provides an foundation to explain earlier simulation findings by Murray et al. (2006), who demonstrated that $\widehat{\Delta}$ had negligible bias when the data were simulated from an ANCOVA model with non-normal random effect and/or residual errors.

Under equal randomization of clusters (which is frequently the case in practice), Theorem 1(b) implies that the model-based variance estimator is also robust to model misspecification. In other words, the standard error estimates returned by standard software for fitting linear mixed models yields (asymptotically) correct uncertainty statements. Taken together, the robustness of the point estimator and variance estimator implies that model-based inference via mixed-model ANCOVA, e.g. the p-value and confidence interval output by standard statistical software, are asymptotically valid without requiring any parametric assumptions on the distribution of (Y, A, \mathbf{X}) .

Under unequal randomization ($\pi \neq 0.5$), the model-based variance estimator may be biased. In this case, we define the sandwich variance estimator of $\widehat{\Delta}$ (following Section 3.2 of Tsiatis, 2007) as the second-row, second-column entry of

$$\left\{ \sum_{i=1}^m \mathbf{Q}_i^{o\top} \widehat{\Sigma}_i^{-1} \mathbf{Q}_i^o \right\}^{-1} \left\{ \sum_{i=1}^m \mathbf{Q}_i^{o\top} \widehat{\Sigma}_i^{-1} (\mathbf{Y}_i^o - \mathbf{Q}_i^o \widehat{\boldsymbol{\beta}}) (\mathbf{Y}_i^o - \mathbf{Q}_i^o \widehat{\boldsymbol{\beta}})^\top \widehat{\Sigma}_i^{-1} \mathbf{Q}_i^o \right\} \left\{ \sum_{i=1}^m \mathbf{Q}_i^{o\top} \widehat{\Sigma}_i^{-1} \mathbf{Q}_i^o \right\}^{-1},$$

where $\mathbf{Y}_i^o = (Y_{i,j_1}, \dots, Y_{i,j_{N_i}})$ is the vector of observed outcomes and $\widehat{\boldsymbol{\beta}} = (\widehat{\beta}_0, \widehat{\beta}_A, \widehat{\boldsymbol{\beta}}_{\mathbf{X}}^\top)^\top$. Given our Assumptions 1 and 2, the sandwich variance estimator is consistent to v for all $\pi \in (0, 1)$ and becomes asymptotically equivalent to the model-based variance estimator if the mixed-model ANCOVA model is correctly specified.

Intuitively, Theorem 1 is proved by “translating” a CRT to an unit-randomized trial, where each unit is a cluster and the observations collected within a unit are akin to repeatedly measured outcomes of the unit. Since all repeat measures are identically distributed

according to Assumption 1(b), the average treatment effect across the repeat measures is identical to Δ^* . When each cluster has a different number of enrolled individuals, we conceptualize it as a missing data problem where M_{ij} is the non-missingness indicator, in which case the non-informative recruitment condition (Assumption 2) corresponds to missingness completely at random (Rubin, 1976). Based on this conceptualization, Theorem 1 is then proved by invoking the asymptotic results in van der Vaart (1998); a complete proof along with the explicit influence function of $\hat{\Delta}$ is provided in the Supplementary Material.

3.2 Potential Precision Gain from Covariate Adjustment

In CRTs, covariate adjustment by mixed-model ANCOVA may reduce precision compared to no covariate adjustment, even under equal randomization. This finding is in sharp contrast to existing results for individually-randomized trials, where covariate adjustment by ANCOVA does not harm the asymptotic efficiency under equal randomization. Heuristically, the efficiency loss of mixed-model ANCOVA can occur if we misspecify the true covariance structure, which can be different from the assumed compound symmetric correlation structure in mixed-model ANCOVA; such misspecification will compromise the ability of $\hat{\beta}_{\mathbf{X}}$ in capturing the true relationship between $\sum_{j \in \mathcal{O}_i} Y_{ij}$ and $\sum_{j \in \mathcal{O}_i} \mathbf{X}_{ij}$, which can then inflate the variance of $\hat{\Delta}$. We illustrate this result in the ensuing simulation study, where the mixed-model ANCOVA estimator can have 4% to 37% larger variance than the unadjusted estimator, under misspecification of its working model.

To improve precision by covariate adjustment, we further consider a cluster-level ANCOVA model based on cluster-specific means aggregated from individual-level information. For $i = 1, \dots, m$, let $\bar{Y}_i^o = N_i^{-1} \sum_{j \in \mathcal{O}_i} Y_{ij}$ and $\bar{\mathbf{X}}_i^o = N_i^{-1} \sum_{j \in \mathcal{O}_i} \mathbf{X}_{ij}$, the cluster-level ANCOVA working model is defined as

$$E[\bar{Y}_i^o | A_i, \bar{\mathbf{X}}_i^o] = \alpha_0 + \alpha_A A_i + \boldsymbol{\alpha}_{\bar{\mathbf{X}}^o}^\top \bar{\mathbf{X}}_i^o. \quad (2)$$

Under model (2), Δ^* is estimated by the ordinary least square estimator of α_A , which we refer to as $\hat{\Delta}^{(cl)}$. Under Assumption 1, it is straightforward to infer from Yang and Tsiatis

(2001) that $\widehat{\Delta}^{(\text{cl})}$ is robust and improves precision by adjusting for prognostic covariates when $\pi = 0.5$; under the same condition, we can infer from Wang et al. (2019) that the model-based variance estimator under $\widehat{\Delta}^{(\text{cl})}$ is also valid without requiring model (2) to be correctly specified. In CRTs with an equal number of enrolled individuals per cluster, we show below that covariate adjustment by cluster-level ANCOVA leads to equal or even more precision gain than covariate adjustment by mixed-model ANCOVA, assuming that $\pi = 0.5$.

Theorem 2. *Suppose $\widehat{\Delta}$ and $\widehat{\Delta}^{(\text{cl})}$ are two estimators for Δ^* based on the same set of covariates \mathbf{X} . Under Assumptions 1, 2, and assuming a balanced design with $N_i = \tilde{n}$ for all i and $\pi = 0.5$, the asymptotic variance of $\widehat{\Delta}$ is larger than or equal to the asymptotic variance of $\widehat{\Delta}^{(\text{cl})}$. Their asymptotic variances are identical if and only if*

$$\text{Var}(\overline{\mathbf{X}}^o)^{-1}\text{Cov}(\overline{\mathbf{X}}^o, \overline{Y}^o) = \text{Var}(\mathbf{X})^{-1}\text{Cov}(\mathbf{X}, Y). \quad (3)$$

Equation (3) holds when the mixed-model ANCOVA is correctly specified, data from each individual within a cluster are not correlated, or \mathbf{X} only contain cluster-level covariates. Beyond these three special cases, Equation (3) may not hold, suggesting the cluster-level ANCOVA estimator has higher asymptotic efficiency than the mixed-model ANCOVA estimator in a CRT. Of note, by collapsing outcome observations at the cluster level, the cluster-level ANCOVA obviates the need to model the intracluster correlation structure, whereas the mixed-model ANCOVA imposes a parametric random intercept to induce the compound symmetric intracluster correlation structure. Intuitively, when the true intracluster correlation structure is not compound symmetric, the ability of using individual-level covariates to explain the cluster-level outcome variation can be compromised, which may lead to reduced precision gain or even precision loss compared to no adjustment in $\widehat{\Delta}$.

If the cluster sizes vary across clusters, the efficiency comparison between mixed-model and cluster-level ANCOVA is generally indeterminate. By increasing the variation of cluster sizes, the precision of both estimators tends to decrease, but by a different amount. In the special case where the mixed-model ANCOVA is correctly specified, the mixed-model ANCOVA estimator is efficient by the theory of maximum likelihood and provides higher

precision than the cluster-level ANCOVA estimator. Otherwise, their efficiency comparison depends on the degree of cluster sizes variability, magnitude of intracluster correlation, and the prognostic value of covariates. In the ensuing simulation study and data application, we give examples to demonstrate that the mixed-model ANCOVA estimator can be either more precise or less precise than the cluster-level ANCOVA estimator, under different sets of design parameters.

3.3 Extensions to Stratified Randomization

Stratified randomization refers to a restricted randomization procedure that achieves between-group balance on certain covariates within each pre-specified stratum, and has also been frequently used in CRTs to minimize chance imbalance (Ivers et al., 2012). For each cluster i , let S_i be a categorical variable that encodes the randomization strata \mathcal{S} . For example, if cluster randomization is stratified by geographical location (urban versus rural), then the randomization strata are $\mathcal{S}=\{\text{urban, rural}\}$ and $S_i \in \mathcal{S}$. We assume that the number of strata is fixed and the randomization proportion within strata remains $\pi \in (0, 1)$. Under stratified randomization, Assumption 1(c) no longer holds since (A_1, \dots, A_n) are correlated and are further correlated with (S_1, \dots, S_n) . However, Theorem 3 implies that the mixed-model ANCOVA estimator retains its asymptotic validity and that stratified randomization can improve its precision. In addition, the model-based inference remains valid as long as $\pi = 0.5$ and provided that the strata variables are adjusted in the mixed-model ANCOVA as dummy variables.

Theorem 3. (a) Given Assumption 1(a)–(b), stratified randomization, and Assumption 2, the mixed-model ANCOVA estimator $\hat{\Delta}$ for Δ^* is consistent and asymptotically normal with asymptotic variance \tilde{v} under arbitrary misspecification of its working model. (b) Furthermore, under arbitrary model misspecification, $\tilde{v} \leq v$, where v is defined in Theorem 1 as the asymptotic variance of $\hat{\Delta}$ under simple randomization. If $\pi = 0.5$ and S_i is adjusted for in the mixed-model ANCOVA as cluster-level dummy variables, then $\tilde{v} = v$.

Of note, a similar result can be stated for the cluster-level ANCOVA estimator, $\widehat{\Delta}^{(\text{cl})}$, as inferred by Corollary 1 of Wang et al. (2021). The efficiency comparison between $\widehat{\Delta}$ and $\widehat{\Delta}^{(\text{cl})}$ under $\pi = 0.5$ and stratified randomization therefore follows Section 3.2. We omit the formal statements for brevity.

4 Simulation Study

4.1 Simulation Design

We report a simulation study to demonstrate our main results, including: (i) the mixed-model ANCOVA estimator is robust under simple or stratified randomization, (ii) covariate adjustment by mixed-model ANCOVA can lead to precision loss, (iii) mixed-model ANCOVA is less efficient than cluster-level ANCOVA under equal cluster sizes, and (iv) a correctly specified mixed-model ANCOVA can lead to smaller variance than cluster-level ANCOVA under variable cluster sizes. We consider three scenarios with different data generating processes, where the aims (ii), (iii), and (iv) are pursued by scenarios 1, 2, and 3, respectively; Aim (i) is demonstrated by all three scenarios. In each scenario, we study both the small-sample and large-sample behaviors of the estimators by setting $m = 20$ and $m = 200$.

In Scenario 1, we generate the treatment indicator $A_i \sim \text{Bernoulli}(\pi = 0.5)$ and simulate the covariate $X_{ij} \sim N(0, 4)$ for each $j = 1, \dots, n$ in cluster i . For each individual, we assume $Y_{ij} = X_{ij} - n^{-1} \sum_{k=1}^n X_{ik} + \delta_i + \epsilon_{ij}$, where $\delta_i \sim N(0, 1)$ and $\epsilon_{ij} \sim N(0, 25)$. The random variables $\{A_i, X_{i1}, \dots, X_{in}, \delta_i, \epsilon_{i1}, \dots, \epsilon_{in}\}$ are mutually independent and also independent across i . For generating the observed data, we independently draw N_i from a uniform distribution on $\{4, \dots, 12\}$ and then sample N_i data vectors from $\{(Y_{ij}, A_i, X_{ij}) : j = 1, \dots, n\}$ without replacement. For Scenario 2, we consider a fixed cluster size with $N_i = 8$ and stratified randomization. For each cluster i , we first independently generate a binary strata variable $S_i \sim \text{Bernoulli}(0.6)$ and then assign A_i under stratified randomization with each stratum defined by S_i and let $\pi = 0.5$. We then generate the observed outcome Y_{ij} from $Y_{ij} = 2S_i(A_i + X_{ij} + n^{-1} \sum_{k=1}^n X_{ik}) + \epsilon_{ij}$, where X_{ij} and ϵ_{ij} are as defined as in Scenario

1. Similarly, N_i observed data vectors are drawn from $\{(Y_{ij}, A_i, X_{ij}) : j = 1, \dots, n\}$ without replacement. The data generating process for scenario 3 is the same as Scenario 1 except that the outcome distribution conditional on A_i, X_{ij} is $Y_{ij} = X_{ij} + \delta_i + \epsilon_{ij}$, which will be identical to the mixed-model ANCOVA used for analyzing the data with a prognostic covariate X_{ij} . The marginal intraclass correlation coefficient among outcomes is 0.02, 0.09, and 0.03 for Scenarios 1 to 3 respectively, representing a mild correlation among individuals in the same cluster.

For all scenarios, we simulate 10,000 data sets. We estimate the average treatment effect (the true Δ^* is 0 for Scenarios 1 and 3 and 1.2 for Scenario 2) by the mixed-model unadjusted estimator, the mixed-model ANCOVA estimator, as well as the cluster-level ANCOVA estimator. The mixed-model unadjusted estimator is obtained from the mixed-model ANCOVA estimator but with no adjustment for covariates. Both the mixed-model and cluster-level ANCOVA models adjust for X in Scenarios 1 and 3 and further $\{X, S\}$ in Scenario 2. For each estimator, we consider the following performance metrics: bias, empirical standard error, averaged model-based standard error, coverage probability of the 95% confidence intervals (constructed using a normal approximation and the model-based standard error), and relative efficiency *vs.* the mixed-model unadjusted estimator.

4.2 Simulation Results

Table 1 summarizes the simulation results. All estimators have negligible bias and nominal coverage probability for the true average treatment effect across Scenarios 1 to 3. Matching our analytical derivations, the mixed-model ANCOVA estimator is unbiased in Scenarios 1 and 2, where its working model is misspecified, and in Scenario 2, when stratified randomization is considered. With a larger number of clusters ($m = 200$), the model-based standard error for mixed-model ANCOVA matches the empirical standard error. When $m = 20$, all estimators have 0-3% under-coverage due to the finite-sample bias of normal-based confidence intervals in CRTs; the under-coverage can be alleviated, for example, by considering a t -distribution with heavier tails.

Table 1: Simulation results for Scenarios 1–3 with 20 or 200 clusters. The performance metrics are bias, empirical standard error (Emp SE), averaged model-based standard error (ASE), coverage probability of 95% confidence intervals based on normal approximation and model-based standard error (CP), and relative efficiency to the mixed-model unadjusted estimator (RE). Across all scenarios and estimators, the maximum Monte Carlo standard errors for bias, Emp SE, ASE, CP, RE are 0.013, 0.009, 0.010, 0.003, 0.009, respectively.

		Estimator	Bias	Emp SE	ASE	CP	RE
Scenario 1	$m = 20$	mixed-model unadjusted	0.00	0.95	1.00	0.94	1.00
		mixed-model ANCOVA	-0.01	0.97	1.01	0.93	0.96
		cluster-level ANCOVA	0.00	1.00	1.00	0.94	0.91
	$m = 200$	mixed-model unadjusted	0.01	0.29	0.30	0.95	1.00
		mixed-model ANCOVA	0.01	0.30	0.30	0.95	0.96
		cluster-level ANCOVA	0.01	0.30	0.30	0.95	0.94
Scenario 2	$m = 20$	mixed-model unadjusted	-0.02	1.29	1.29	0.93	1.00
		mixed-model ANCOVA	0.00	1.09	1.09	0.92	1.40
		cluster-level ANCOVA	0.01	1.05	1.03	0.93	1.51
	$m = 200$	mixed-model unadjusted	-0.01	0.41	0.41	0.95	1.00
		mixed-model ANCOVA	0.00	0.34	0.34	0.95	1.40
		cluster-level ANCOVA	0.00	0.32	0.33	0.95	1.58
Scenario 3	$m = 20$	mixed-model unadjusted	-0.01	1.00	1.02	0.94	1.00
		mixed-model ANCOVA	-0.01	0.95	0.99	0.94	1.11
		cluster-level ANCOVA	-0.01	1.00	0.98	0.94	1.00
	$m = 200$	mixed-model unadjusted	0.01	0.31	0.31	0.95	1.00
		mixed-model ANCOVA	0.01	0.29	0.29	0.95	1.12
		cluster-level ANCOVA	0.01	0.30	0.30	0.95	1.05

Results under Scenario 1 demonstrate that the mixed-model ANCOVA estimator can be less efficient than the mixed-model unadjusted estimator. Specifically, the data generating distribution under Scenario 1 implies that $Cov(\bar{Y}^o, \bar{X}^o) = 0$ but $Cov(Y, X) = 0.875$, which means the aggregated covariate is not prognostic at the cluster level but is prognostic at the individual level. Since the treatment is assigned at the cluster level, only $Cov(\bar{Y}^o, \bar{X}^o)$ is related to possible variance reduction in estimating the average treatment effect. For Scenario 1, $Cov(\bar{Y}^o, \bar{X}^o) = 0$ implies that covariate adjustment provides no variance reduction. In contrast, mixed-model ANCOVA exploits both $Cov(\bar{Y}^o, \bar{X}^o)$ and $Cov(Y, X)$ for estimating β_X and therefore leads to 4% efficiency loss by tapping into correlations that are ancillary to the estimation of cluster-level treatment effect. The magnitude of efficiency loss depends on the variance of X and can be as high as 37% if the variance of X is increased to 100 (for fixed β_X). In this scenario, the cluster-level ANCOVA is also less efficient than the mixed-model unadjusted estimator, since the unadjusted mixed-model can be considered correctly specified (by marginalizing over X) and the variation in cluster sizes results in further efficiency loss of the cluster-level ANCOVA estimator as discussed in Section 3.2.

In Scenario 2, both the mixed-model ANCOVA and cluster-level ANCOVA improve precision from covariate adjustment, with the former 18% less efficient than the latter. Similar to Scenario 1, mixed-model ANCOVA fails to accurately estimate the $Cov(\bar{Y}^o, \bar{X}^o)$ and therefore covariate adjustment only achieves partial variance reduction. Finally, the data generating distribution for Scenario 3 is compatible with the assumptions of mixed-model ANCOVA. The mixed-model ANCOVA estimator, as a result, has the smallest empirical standard error among the three estimators. Similar to Scenario 1, the cluster-level ANCOVA estimator is prone to efficiency loss when the cluster size varies, and demonstrates 7% variance inflation compared to the mixed-model ANCOVA estimator; their difference in variance can become smaller with a decreasing variation in cluster sizes.

To further compare the estimators given non-normal data and large intracluster correlations, we repeat the above simulation study with a modification on the data generating distribution. For Scenarios 1-3, we add an independent random effect γ_i that follows a

Gamma distribution with $E[\gamma_i] = Var(\gamma_i) = 25$; all other specifications remain unchanged. The marginal intracluster correlation coefficient now becomes 0.47, 0.45, and 0.47 for Scenarios 1 to 3, respectively. The simulation results are summarized in Table 2 below, which shows similar results to Table 1: all estimators remain robust and the precision comparisons are unchanged. The major difference from the first simulation study is the comprised precision gain from covariate adjustment in Scenarios 2 and 3, a natural result from introducing a larger random effect.

5 Applications to Three Cluster-Randomized Trials

5.1 Background and Contexts

Task Shifting and Blood Pressure Control in Ghana (TASSH) is a CRT evaluating the effectiveness of a nurse-led task shifting strategy for hypertension control through systolic blood pressure (SBP) reduction (Ogedegbe et al., 2018). Thirty-two community health centers were randomly assigned to receive treatment (provision of health insurance coverage plus TASSH, 389 patients within 16 clusters) or usual care (provision of health insurance coverage only, 368 patients within 16 clusters). Each cluster has recruited a different number of individuals, ranging from 17 to 31. We focus on the primary outcome of the study, change in SBP from baseline to 12 months; the included baseline covariates are age, SBP, Diastolic Blood Pressure, and the location of the health center (rural or urban).

Improving Early Childhood Development in Zambia (IECDZ) is a CRT assessing the effect of a community-based early childhood development (ECD) program on physical and cognitive development (Rockers et al., 2018). Thirty clusters of villages were equally randomized to receive treatment (ECD, 195 caregiver-child dyads within 15 clusters) or control (no intervention, 182 caregiver-child dyads within 15 clusters) with each cluster including 2–26 caregiver-child dyads. We focus on the continuous outcome, height-for-age z-score (HAZ), at the year-2 follow-up, which was used to determine children’s stunting status ($HAZ < -2$) in the primary analysis of the study. We adjust for the baseline covariates age, baseline HAZ,

Table 2: Simulation results for modified Scenarios 1–3 given a non-normal distribution with large intracluster correlation. The performance metrics are bias, empirical standard error (Emp SE), averaged model-based standard error (ASE), coverage probability of 95% confidence intervals based on normal approximation and model-based standard error (CP), and relative efficiency to the mixed-model unadjusted estimator (RE). Across all scenarios and estimators, the maximum Monte Carlo standard errors for bias, Emp SE, ASE, CP, RE are 0.026, 0.018, 0.001, 0.003, 0.009, respectively.

		Estimator	Bias	Emp SE	ASE	CP	RE
Scenario 1	$m = 20$	mixed-model unadjusted	0.03	2.50	2.46	0.92	1.00
		mixed-model ANCOVA	0.03	2.50	2.54	0.92	1.00
		cluster-level ANCOVA	0.04	2.57	2.53	0.94	0.95
	$m = 200$	mixed-model unadjusted	-0.01	0.77	0.77	0.97	1.00
		mixed-model ANCOVA	0.00	0.78	0.77	0.95	0.99
		cluster-level ANCOVA	-0.01	0.78	0.77	0.95	0.99
Scenario 2	$m = 20$	mixed-model unadjusted	0.02	2.56	2.55	0.92	1.00
		mixed-model ANCOVA	0.04	2.48	2.45	0.94	1.07
		cluster-level ANCOVA	0.05	2.52	2.50	0.93	1.03
	$m = 200$	mixed-model unadjusted	0.00	0.82	0.82	0.95	1.00
		mixed-model ANCOVA	0.00	0.79	0.79	0.95	1.08
		cluster-level ANCOVA	0.00	0.78	0.78	0.95	1.11
Scenario 3	$m = 20$	mixed-model unadjusted	-0.01	2.53	2.47	0.92	1.00
		mixed-model ANCOVA	-0.01	2.50	2.52	0.92	1.02
		cluster-level ANCOVA	-0.02	2.59	2.53	0.93	0.95
	$m = 200$	mixed-model unadjusted	0.00	0.77	0.77	0.95	1.00
		mixed-model ANCOVA	0.01	0.76	0.77	0.95	1.02
		cluster-level ANCOVA	0.01	0.77	0.77	0.95	1.01

as well as child motor score.

The *Work, Family, and Health Study* (WFHS) is a CRT designed to enhance the understanding of the impact of workplace practices and policies on employees’ work, family, and health outcomes ([Work, Family, and Health Study \(WFHS\), 2018](#)). We use data from one study site, a Fortune 500 company, where 56 study groups were randomly assigned to receive a workplace intervention (423 employees in 29 clusters) or usual practice (400 employees in 27 clusters) with each cluster including 3–50 employees. We focus on the control over work hours (CWH) at the 6-month follow-up, which is a continuous measure ranging from 1 to 5 and demonstrated the largest treatment effect ([Kelly et al., 2014](#)). Baseline CWH, job function (core or supporting), and cluster size are adjusted for as baseline covariates.

5.2 Data Analysis Results

In each CRT, we estimate the treatment effect using the mixed-model unadjusted estimator, mixed-model ANCOVA estimator, and cluster-level ANCOVA estimator. For illustration purposes, we assume that simple randomization is used in all CRTs. Furthermore, individuals with missing outcomes (15%, 1%, and 20% for TASSH, IECDZ, and WFHS) are removed from the analysis and missing baseline variables are imputed once by the mean of non-missing observations.

Table 3 presents the data analysis results. For the TASSH study, covariate adjustment by the cluster-level ANCOVA results in a 10% variance reduction and a 5% narrower confidence interval compared to the mixed-model unadjusted estimator. In contrast, the mixed-model ANCOVA estimator only has a slightly larger variance estimate than the mixed-model unadjusted estimator, which may suggest a small efficiency loss due to covariate adjustment in the presence of a misspecified intracluster correlation structure.

For the IECDZ study, the mixed-model unadjusted estimator has the smallest variance, which resembles findings from Scenario 1 of our simulation study. Possible reasons for efficiency loss of the cluster-level ANCOVA estimator are that covariates are not prognostic and the cluster sizes are moderately variable (coefficient of variation of cluster sizes is 0.41).

Table 3: Summary of data analyses results: point estimate of the average treatment effect (Est), model-based estimator for standard error (SE), 95% confidence interval (CI), and proportion variance reduction compared to the unadjusted estimator (PVR). Positive (negative) PVR indicates that covariate adjustment leads to variance reduction (inflation).

Study name	Estimators	Est	SE	95% CI	PVR
TASSH	mixed-model unadjusted	-1.29	2.08	(-5.36, 2.78)	-
	mixed-model ANCOVA	-2.22	2.09	(-6.32, 1.87)	-1%
	cluster-level ANCOVA	-1.54	1.97	(-5.40, 2.33)	10%
IECDZ	mixed-model unadjusted	0.08	0.14	(-0.19, 0.35)	-
	mixed-model ANCOVA	0.08	0.15	(-0.22, 0.37)	-21%
	cluster-level ANCOVA	0.08	0.14	(-0.20, 0.36)	-8%
WFHS	mixed-model unadjusted	0.16	0.07	(0.01, 0.30)	-
	mixed-model ANCOVA	0.21	0.05	(0.12, 0.31)	56%
	cluster-level ANCOVA	0.25	0.05	(0.15, 0.36)	47%

Compared to cluster-level ANCOVA, mixed-model ANCOVA is 13% less efficient for estimating the average treatment effect, which again might be attributed to its misspecification of the intracluster correlation structure.

In the analysis of WFHS, both mixed-model and cluster-level ANCOVA have substantial precision gain compared to an unadjusted analysis, likely because the baseline CWH is highly prognostic of the follow-up outcome. In this example, we observe that mixed-model ANCOVA can return a more efficient average treatment effect estimator than cluster-level ANCOVA. The advantage of mixed-model ANCOVA in the analysis of WFHS can also be because the intracluster correlation structure is close to compound symmetry and that the cluster sizes are highly variable (coefficient of variation of cluster sizes is 0.59).

6 Discussion

Although the mixed-model ANOVA is a standard and commonly used approach to estimate the average treatment effect in CRTs, to date there has been no formal investigation on its asymptotic properties when model assumptions do not hold. In this context, our primary contribution is to establish the consistency and asymptotic normality of the mixed-model ANCOVA estimator under arbitrary misspecification of its working model. Under equal randomization ($\pi = 0.5$), we further prove that the model-based variance estimator remains consistent and henceforth the standard error estimate returned by current software routines yields asymptotically correct uncertainty statements, even if the working model is incorrect. This robustness property is reassuring, and serves to provide new justifications for conducting mixed-model ANCOVA analysis of CRTs.

However, we find interesting caveats on efficiency for mixed-model ANCOVA analysis of CRTs. In contrast to findings in individually-randomized trials, covariate adjustment via mixed-model ANCOVA does not always guarantee a more efficient average treatment effect estimator. When the cluster sizes are constant, the cluster-level ANCOVA model even dominates the mixed-model ANCOVA in terms of efficiency for estimating the average treatment

effect, under arbitrary model misspecifications. The efficiency results under variable cluster sizes are generally indeterminate. Therefore, from an efficiency perspective, the cluster-level ANCOVA estimator, whenever feasible, may be the preferred approach if the cluster sizes are equal or only mildly variable. On the other hand, when the cluster sizes are highly variable, mixed-model ANCOVA estimator can be more efficient provided there is no or only mild model misspecification. In any case, we still maintain the usual recommendation in individually-randomized trials to pre-specify and adjust for baseline covariates that are anticipated to be prognostic of the outcome in CRTs (rather than any covariates that happen to be measured). Finally, our efficiency results are restricted to a balanced design with $\pi = 0.5$. Under unequal randomization, even though mixed-model and cluster-level ANCOVA estimators are still robust to model misspecification, they may not even provide variance reduction relative to the unadjusted estimator. In this case, a potential solution is to include treatment-by-covariates interactions within the cluster-level ANCOVA model, which leads to no asymptotic efficiency loss (Tsiatis et al., 2008; Ye et al., 2020). The empirical performance of this estimator in CRTs, however, is a topic for future research.

Based on the mixed-model ANCOVA, an alternative approach for estimation is through maximizing the restricted maximum likelihood (REML), which is known to reduce the bias of the variance component estimators. If the mixed-model ANCOVA is correctly specified, the maximum likelihood estimator and REML estimator are asymptotically equivalent (c.f. p.17 in Jiang, 2017). We have replicated the simulation study and data applications using the REML estimator in the Supplementary Material. In our simulations, the REML estimator demonstrates slighter better performance than the maximum likelihood estimator in terms of coverage when the number of clusters is small ($m = 20$), whereas these two approaches present negligible differences with a larger number of clusters. In our data applications, the REML variance estimator tends to be smaller than the maximum likelihood variance estimator, especially when the covariates do not appear prognostic. The limited empirical evidence sheds light on the robustness of the REML estimator, although a formal proof of its robustness under arbitrary model misspecification is subject to additional research.

Our results rest on a key identification assumption on non-informative enrollment, violations to which may render the mixed-model ANCOVA estimator biased for estimating the average treatment effect in CRTs. When the cluster sizes are informative, namely, N_i is no longer independent of the outcomes, treatment or covariates (Seaman et al., 2014), the average treatment effect among the enrolled individuals is no longer representative of the whole source population of interest (n individuals of each cluster). For example, consider two clusters with an equal population size but opposite cluster-specific treatment effects. If N_i is larger in the cluster where the treatment is beneficial, then the average treatment effect across all enrolled individuals will be positive, even though the average treatment effect among the whole population of the two clusters remains null. Similarly, when selective recruitment of individuals leads to selection bias, Li et al. (2021) has shown that covariate adjustment via mixed-model ANCOVA is often insufficient for unbiased estimation of the average treatment effect, and implies that valid estimation requires access to baseline information among the non-enrolled population. These practical challenges, in fact, often speak to the inherent limitation of the cluster randomization design, rather than the mixed-model ANCOVA estimator itself. Addressing informative cluster sizes and selection bias in CRTs is beyond the scope of this article, and will be pursued in a separate study.

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Supplementary Material for “On the mixed-model analysis of covariance in cluster-randomized trials”

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In Section **A**, we provide regularity conditions for our theorems; Section **B** proves Theorems 1-3 presented in the main manuscript; and Section **C** compares ML and REML estimation by replicating the simulation study and data application.

A Regularity conditions

We make the following regularity conditions on the estimating function $\psi(\mathbf{Y}, A, \mathbf{X}, \mathbf{M}; \boldsymbol{\theta})$ defined in Equation (1) in Section **B**:

- (1) $\boldsymbol{\theta} \in \Theta$, a compact subset of \mathbb{R}^{p+4} . In addition, $\boldsymbol{\theta} \in \Theta$ implies that $\sigma^2 > 0$.
- (2) There exists a unique $\underline{\boldsymbol{\theta}}$, a inner point of Θ , that satisfies $E[\psi(\mathbf{Y}, A, \mathbf{X}, \mathbf{M}; \underline{\boldsymbol{\theta}})] = \mathbf{0}$.
- (3) The estimating function has finite second moment, $E[|\psi(\mathbf{Y}, A, \mathbf{X}, \mathbf{M}; \underline{\boldsymbol{\theta}})|^2] < \infty$.
- (4) $E\left[\frac{\partial}{\partial \boldsymbol{\theta}} \psi(\mathbf{Y}, A, \mathbf{X}, \mathbf{M}; \boldsymbol{\theta})\Big|_{\boldsymbol{\theta}=\underline{\boldsymbol{\theta}}}\right]$ exists and is invertible.

(5) Let $\psi_k(\mathbf{Y}, A, \mathbf{X}, \mathbf{M}; \boldsymbol{\theta})$ denote the k -th entry of $\boldsymbol{\psi}(\mathbf{Y}, A, \mathbf{X}, \mathbf{M}; \boldsymbol{\theta})$. There exists an integrable function $v(\mathbf{Y}, A, \mathbf{X}, \mathbf{M})$ such that, for $k = 1, \dots, p + 4$,

$$\left\| \frac{\partial}{\partial \boldsymbol{\theta} \partial \boldsymbol{\theta}^\top} \psi_k(\mathbf{y}, a, \mathbf{x}, \mathbf{m}; \boldsymbol{\theta}) \right\| \leq v(\mathbf{y}, a, \mathbf{x}, \mathbf{m})$$

for every $\boldsymbol{\theta}$ in a neighborhood of $\underline{\boldsymbol{\theta}}$ and every $(\mathbf{y}, a, \mathbf{x}, \mathbf{m})$ in the support of $(\mathbf{Y}, A, \mathbf{X}, \mathbf{M})$.

Given Assumptions 1-2 in the main manuscript, the expectation E considered in the regularity conditions (2)-(4) are taken with respect to the joint distribution of $\mathcal{P} = (\mathcal{P}^{(\mathbf{W})}, \mathcal{P}^{(A)}, \mathcal{P}^{(M)})$, which is well defined since they are independent of each other. Under stratified randomization (Section 3.3), since $\mathcal{P}^{(A)}$ is not defined, the expectation E is taken with respect to the observed data distribution \mathcal{P}^* on $(\mathbf{Y}^o, A, \mathbf{X})$, which leads to the same set of regularity conditions but under \mathcal{P}^* rather than \mathcal{P} . The formal characterization of \mathcal{P}^* can be found in Lemmas 3 and 4 of the supplementary material of Wang et al. (2021), and is therefore not reproduced here for brevity.

B Proofs

B.1 Proof of Theorem 1

Proof. For each cluster i , let $j_{i,1} < \dots < j_{i,N_i}$ be the ordered list of indices such that the observed outcomes are $\mathbf{Y}_i^o = (Y_{i,j_{i,1}}, \dots, Y_{i,j_{i,N_i}})$. We define $\mathbf{D}_{M_i} = [\mathbf{e}_{j_{i,1}} \ \mathbf{e}_{j_{i,2}} \ \dots \ \mathbf{e}_{j_{i,N_i}}] \in \mathbb{R}^{n \times N_i}$, where $\mathbf{e}_j \in \mathbb{R}^n$ has the j -th entry 1 and the rest 0. For \mathbf{D}_{M_i} , we use the subscript M_i to indicate that it is a deterministic function of M_i . Then we have $\mathbf{Y}_i^o = \mathbf{D}_{M_i}^\top \mathbf{Y}_i$, $\mathbf{X}_i^o = \mathbf{D}_{M_i}^\top \mathbf{X}_i$, $\mathbf{1}_{N_i} = \mathbf{D}_{M_i}^\top \mathbf{1}_n$ and $\mathbf{D}_{M_i} \mathbf{1}_{N_i} = M_i$, where $\mathbf{X}_i = (\mathbf{X}_{i1}, \dots, \mathbf{X}_{in})^\top$.

The mixed-model ANCOVA for the population of interest can be re-written in matrix notation as:

$$\mathbf{Y}_i = \beta_0 \mathbf{1}_n + A \beta_A \mathbf{1}_n + \mathbf{X}_i \boldsymbol{\beta}_X + \delta_i \mathbf{1}_n + \boldsymbol{\epsilon}_i,$$

where $\mathbf{Y}_i = (Y_{i1}, \dots, Y_{in})^\top$, $\mathbf{X}_i = (\mathbf{X}_{i1}, \dots, \mathbf{X}_{in})^\top \in \mathbb{R}^{n \times p}$, $\boldsymbol{\epsilon}_i = (\epsilon_{i1}, \dots, \epsilon_{in})^\top$. Marginaliz-

ing over the distribution of random effects, we have, given the mixed-model ANCOVA

$$\mathbf{Y}_i | (A_i, \mathbf{X}_i) \sim N(\mathbf{Q}_i \boldsymbol{\beta}, \boldsymbol{\Sigma}),$$

where $\mathbf{Q}_i = (\mathbf{1}_n, A_i \mathbf{1}_n, \mathbf{X}_i) \in \mathbb{R}^{n \times (p+2)}$, $\boldsymbol{\beta} = (\beta_0, \beta_A, \boldsymbol{\beta}_X^\top)^\top \in \mathbb{R}^{p+2}$ and $\boldsymbol{\Sigma} = \sigma^2 \mathbf{I}_n + \tau^2 \mathbf{1}_n \mathbf{1}_n^\top$. We denote the parameters in the above model as $\boldsymbol{\theta} = (\boldsymbol{\beta}^\top, \sigma^2, \tau^2)^\top \in \mathbb{R}^{p+4}$. Then, for the observed outcome, we have $\mathbf{Y}_i^o | (A_i, \mathbf{X}_i^o, N_i) \sim N(\mathbf{D}_{M_i}^\top \mathbf{Q}_i \boldsymbol{\beta}, \mathbf{D}_{M_i}^\top \boldsymbol{\Sigma} \mathbf{D}_{M_i})$ under the mixed-model ANCOVA working model and Assumption 2. We note that, although M_i is not observed, $\mathbf{D}_{M_i}^\top \mathbf{Q}_i = (\mathbf{1}_{N_i}, A_i \mathbf{1}_{N_i}, \mathbf{X}_i^o)$ and $\mathbf{D}_{M_i}^\top \boldsymbol{\Sigma} \mathbf{D}_{M_i} = \sigma^2 \mathbf{I}_{N_i} + \tau^2 \mathbf{1}_{N_i} \mathbf{1}_{N_i}^\top$ are only functions of observed data $(A_i, \mathbf{X}_i^o, N_i)$, which allows the maximum likelihood estimator (MLE) to be well-defined.

Based on the observed data, the log-likelihood function conditioning on $\{A_i, \mathbf{X}_i, N_i\}$ is defined as

$$\begin{aligned} l(\boldsymbol{\theta}; \{\mathbf{Y}_i^o\}_{i=1}^m | \{A_i, \mathbf{X}_i, N_i\}_{i=1}^m) \\ &= C - \frac{1}{2} \sum_{i=1}^m \left\{ \log(|\mathbf{D}_{M_i}^\top \boldsymbol{\Sigma} \mathbf{D}_{M_i}|) + (\mathbf{Y}_i^o - \mathbf{D}_{M_i}^\top \mathbf{Q}_i \boldsymbol{\beta})^\top (\mathbf{D}_{M_i}^\top \boldsymbol{\Sigma} \mathbf{D}_{M_i})^{-1} (\mathbf{Y}_i^o - \mathbf{D}_{M_i}^\top \mathbf{Q}_i \boldsymbol{\beta}) \right\} \\ &= C - \frac{1}{2} \sum_{i=1}^m \left\{ \log(|\mathbf{D}_{M_i}^\top \boldsymbol{\Sigma} \mathbf{D}_{M_i}|) + (\mathbf{Y}_i - \mathbf{Q}_i \boldsymbol{\beta})^\top \mathbf{D}_{M_i} (\mathbf{D}_{M_i}^\top \boldsymbol{\Sigma} \mathbf{D}_{M_i})^{-1} \mathbf{D}_{M_i}^\top (\mathbf{Y}_i - \mathbf{Q}_i \boldsymbol{\beta}) \right\} \end{aligned}$$

where C is a constant independent of the parameters $\boldsymbol{\theta}$. The derivative of the log-likelihood function is then

$$\frac{\partial l(\boldsymbol{\theta}; \{\mathbf{Y}_i^o\}_{i=1}^m | \{A_i, \mathbf{X}_i, N_i\}_{i=1}^m)}{\partial \boldsymbol{\theta}} = - \sum_{i=1}^m \begin{pmatrix} \mathbf{Q}_i^\top \mathbf{V}_i (\mathbf{Y}_i - \mathbf{Q}_i \boldsymbol{\beta}) \\ -tr(\mathbf{V}_i) + (\mathbf{Y}_i - \mathbf{Q}_i \boldsymbol{\beta})^\top \mathbf{V}_i^2 (\mathbf{Y}_i - \mathbf{Q}_i \boldsymbol{\beta}) \\ -\mathbf{1}_n^\top \mathbf{V}_i \mathbf{1}_n + (\mathbf{Y}_i - \mathbf{Q}_i \boldsymbol{\beta})^\top \mathbf{V}_i \mathbf{1}_n \mathbf{1}_n^\top \mathbf{V}_i (\mathbf{Y}_i - \mathbf{Q}_i \boldsymbol{\beta}) \end{pmatrix},$$

where $\mathbf{V}_i = \mathbf{D}_{M_i} (\mathbf{D}_{M_i}^\top \boldsymbol{\Sigma} \mathbf{D}_{M_i})^{-1} \mathbf{D}_{M_i}^\top \in \mathbb{R}^{n \times n}$ and $tr(\mathbf{V}_i)$ is the trace of \mathbf{V}_i . We hence define the estimating function as

$$\boldsymbol{\psi}(\mathbf{Y}, A, \mathbf{X}, M; \boldsymbol{\theta}) = \begin{pmatrix} \mathbf{Q}^\top \mathbf{V} (\mathbf{Y} - \mathbf{Q} \boldsymbol{\beta}) \\ -tr(\mathbf{V}) + (\mathbf{Y} - \mathbf{Q} \boldsymbol{\beta})^\top \mathbf{V}^2 (\mathbf{Y} - \mathbf{Q} \boldsymbol{\beta}) \\ -\mathbf{1}_n^\top \mathbf{V} \mathbf{1}_n + (\mathbf{Y} - \mathbf{Q} \boldsymbol{\beta})^\top \mathbf{V} \mathbf{1}_n \mathbf{1}_n^\top \mathbf{V} (\mathbf{Y} - \mathbf{Q} \boldsymbol{\beta}) \end{pmatrix}. \quad (1)$$

The MLE for $\boldsymbol{\theta}$ is define as a solution to the estimating equation

$$\sum_{i=1}^n \boldsymbol{\psi}(\mathbf{Y}_i, A_i, \mathbf{X}_i, \mathbf{M}_i; \boldsymbol{\theta}) = \mathbf{0}.$$

We next prove the consistency of $\widehat{\boldsymbol{\beta}}_A$ to Δ^* , under arbitrary misspecification of its working model. By Assumption 1, $\Delta^* = E\{Y_{ij}(1)\} - E\{Y_{ij}(0)\} = E[Y(1)] - E[Y(0)]$. Given the regularity conditions, similar to Example 19.8 of [van der Vaart \(1998\)](#), the estimating function $\boldsymbol{\psi}(\mathbf{Y}, A, \mathbf{X}, \mathbf{M}; \boldsymbol{\theta})$ is Glivenko-Cantelli, and, hence, by Theorem 5.9 of [van der Vaart \(1998\)](#), $\widehat{\boldsymbol{\theta}} \xrightarrow{P} \underline{\boldsymbol{\theta}}$, where $\underline{\boldsymbol{\theta}} = (\underline{\beta}_0, \underline{\beta}_A, \underline{\boldsymbol{\beta}}_X^\top, \underline{\sigma}^2, \underline{\tau}^2)^\top$ solves $E[\boldsymbol{\psi}(\mathbf{Y}, A, \mathbf{X}, \mathbf{M}; \boldsymbol{\theta})] = \mathbf{0}$. To compute $\underline{\beta}_A$, by Assumption 2, $E[\boldsymbol{\psi}(\mathbf{Y}, A, \mathbf{X}, \mathbf{M}; \underline{\boldsymbol{\theta}})] = \mathbf{0}$ implies

$$\begin{aligned} \mathbf{1}_n^\top E[\underline{\mathbf{V}}] E[\mathbf{Y} - \underline{\beta}_0 \mathbf{1}_n - A \underline{\beta}_A \mathbf{1}_n - \mathbf{X}_i^\top \underline{\boldsymbol{\beta}}_X] &= 0, \\ \mathbf{1}_n^\top E[\underline{\mathbf{V}}] E[A(\mathbf{Y} - \underline{\beta}_0 \mathbf{1}_n - A \underline{\beta}_A \mathbf{1}_n - \mathbf{X}_i^\top \underline{\boldsymbol{\beta}}_X)] &= 0, \end{aligned}$$

where $\underline{\mathbf{V}} = \mathbf{D}_M (\mathbf{D}_M^\top \underline{\boldsymbol{\Sigma}} \mathbf{D}_M)^{-1} \mathbf{D}_M^\top$ with $\underline{\boldsymbol{\Sigma}} = \underline{\sigma}^2 \mathbf{I}_n + \underline{\tau}^2 \mathbf{1}_n \mathbf{1}_n^\top$. The above two equations imply that

$$\mathbf{1}_n^\top E[\underline{\mathbf{V}}] E[\mathbf{Y}(1) - \mathbf{Y}(0) - \underline{\beta}_A \mathbf{1}_n] = 0.$$

By Assumption 1, we have $E[\mathbf{Y}(a)] = \mathbf{1}_n E[Y(a)]$ for $a = 0, 1$. Then

$$\mathbf{1}_n^\top E[\underline{\mathbf{V}}] \mathbf{1}_n E[Y(1) - Y(0) - \underline{\beta}_A] = 0.$$

To show $\underline{\beta}_A = E[Y(1)] - E[Y(0)]$, it suffices to prove $E[\mathbf{1}_n^\top \underline{\mathbf{V}} \mathbf{1}_n] > 0$. Direct algebra gives that

$$\mathbf{V} = \mathbf{D}_M \left(\frac{1}{\sigma^2} \mathbf{I}_N - \frac{\tau^2}{\sigma^2(\sigma^2 + N\tau^2)} \mathbf{1}_N \mathbf{1}_N^\top \right) \mathbf{D}_M^\top,$$

which implies that $\mathbf{1}_n^\top \underline{\mathbf{V}} \mathbf{1}_n = \frac{N}{\sigma^2 + N\tau^2}$. Since $N \geq 2$, $\mathbf{1}_n^\top \underline{\mathbf{V}} \mathbf{1}_n > 0$ as long as $\underline{\sigma}^2 > 0$. This is implied by the regularity conditions (1) and (2), which completes the proof of consistency.

We next prove the asymptotic normality. By the regularity conditions, Theorem 5.41 of [van der Vaart \(1998\)](#) implies that

$$\sqrt{n}(\widehat{\boldsymbol{\theta}} - \underline{\boldsymbol{\theta}}) = \frac{1}{\sqrt{m}} \sum_{i=1}^m \mathbf{B}^{-1} \boldsymbol{\psi}(\mathbf{Y}_i, A_i, \mathbf{X}_i, \mathbf{M}_i; \underline{\boldsymbol{\theta}}) + o_p(\mathbf{1}),$$

where $\mathbf{B} = E \left[\frac{\partial}{\partial \boldsymbol{\theta}} \boldsymbol{\psi}(\mathbf{Y}, A, \mathbf{X}, \mathbf{M}; \boldsymbol{\theta}) \Big|_{\boldsymbol{\theta} = \underline{\boldsymbol{\theta}}} \right]$. Then, by computing \mathbf{B}^{-1} , we get

$$\sqrt{n}(\widehat{\Delta} - \underline{\Delta}) = \frac{1}{\sqrt{m}} \sum_{i=1}^m IF(\mathbf{Y}_i, A_i, \mathbf{X}_i, \mathbf{M}_i; \underline{\boldsymbol{\theta}}) + o_p(\mathbf{1}),$$

where

$$IF(\mathbf{Y}, A, \mathbf{X}, \mathbf{M}; \underline{\boldsymbol{\theta}}) = \frac{A - \pi}{\pi(1 - \pi) \mathbf{1}_n^\top E[\mathbf{V}] \mathbf{1}_n} \mathbf{1}_n^\top \mathbf{V}(\mathbf{Y} - \mathbf{Q}\underline{\boldsymbol{\beta}}) \quad (2)$$

is the influence function for $\widehat{\Delta}$, which is also the second component of $\mathbf{B}^{-1} \boldsymbol{\psi}(\mathbf{Y}, A, \mathbf{X}, \mathbf{M}; \boldsymbol{\theta})$. Assumptions 1-2 in the main manuscript imply that $IF(\mathbf{Y}_i, A_i, \mathbf{X}_i, \mathbf{M}_i; \underline{\boldsymbol{\theta}}), i = 1, \dots, m$ are independent and identically distributed. The regularity conditions (3) and (4) imply that the influence function has bounded second moments. Hence, by the Central Limit Theorem, we have $\sqrt{n}(\widehat{\Delta} - \underline{\Delta}) \xrightarrow{d} N(0, v)$ with $v = E[IF^2(\mathbf{Y}, A, \mathbf{X}, \mathbf{M}; \underline{\boldsymbol{\theta}})]$.

To compute the asymptotic variance v , we observe that, by the last equation of $E[\boldsymbol{\psi}(\mathbf{Y}, A, \mathbf{X}, \mathbf{M}; \underline{\boldsymbol{\theta}})] = \mathbf{0}$, we have

$$E[\mathbf{1}_n^\top \mathbf{V}(\mathbf{Y} - \mathbf{Q}\underline{\boldsymbol{\beta}})(\mathbf{Y} - \mathbf{Q}\underline{\boldsymbol{\beta}})^\top \mathbf{V} \mathbf{1}_n] = \mathbf{1}_n^\top E[\mathbf{V}] \mathbf{1}_n. \quad (3)$$

Hence

$$\begin{aligned} v &= \frac{E[(A - \pi)^2 \{\mathbf{1}_n^\top \mathbf{V}(\mathbf{Y} - \mathbf{Q}\underline{\boldsymbol{\beta}})\}^2]}{\pi^2(1 - \pi)^2 (\mathbf{1}_n^\top E[\mathbf{V}] \mathbf{1}_n)^2} \\ &= \frac{1}{(1 - \pi)^2 \mathbf{1}_n^\top E[\mathbf{V}] \mathbf{1}_n} + \frac{(1 - 2\pi) E[A \{\mathbf{1}_n^\top \mathbf{V}(\mathbf{Y} - \mathbf{Q}\underline{\boldsymbol{\beta}})\}^2]}{\pi^2(1 - \pi)^2 (\mathbf{1}_n^\top E[\mathbf{V}] \mathbf{1}_n)^2}. \end{aligned}$$

If $\pi = 0.5$, then the asymptotic variance simply reduces to $4(\mathbf{1}_n^\top E[\mathbf{V}] \mathbf{1}_n)^{-1}$.

Recall the model-based variance estimator for $\widehat{\boldsymbol{\beta}}$ is

$$\widehat{Var}(\widehat{\boldsymbol{\beta}}) = \left(\sum_{i=1}^m \mathbf{Q}_i^\circ \widehat{\boldsymbol{\Sigma}}_i^{-1} \mathbf{Q}_i^\circ \right)^{-1},$$

where $\mathbf{Q}_i^\circ = \mathbf{D}_{M_i}^\top \mathbf{Q}_i = (\mathbf{1}_{N_i}, A_i \mathbf{1}_{N_i}, \mathbf{X}_i^\circ)$ and $\widehat{\boldsymbol{\Sigma}}_i = \frac{m}{m-p-2} (\widehat{\sigma}^2 \mathbf{I}_{N_i} + \widehat{\tau}^2 \mathbf{1}_{N_i} \mathbf{1}_{N_i}^\top)$ with $\widehat{\sigma}^2, \widehat{\tau}^2$ being the MLE for variance components parameters, σ^2, τ^2 , in the mixed-model ANCOVA, respectively. We next show that $m \widehat{Var}(\widehat{\boldsymbol{\beta}}) \xrightarrow{P} (E[\mathbf{Q}^\top \mathbf{V} \mathbf{Q}])^{-1}$. By the Woodbury matrix identity, we have

$$\widehat{\boldsymbol{\Sigma}}_i^{-1} = \frac{m-p-2}{m} \widehat{\sigma}^{-2} \left(\mathbf{I}_{N_i} - \frac{\widehat{\tau}^2}{\widehat{\sigma}^2 + N_i \widehat{\tau}^2} \mathbf{1}_{N_i} \mathbf{1}_{N_i}^\top \right).$$

Using the formula of \mathbf{V} and the result that $\hat{\sigma}^2 = \underline{\sigma}^2 + o_p(1)$ and $\hat{\tau}^2 = \underline{\tau}^2 + o_p(1)$ (as implied by $\hat{\boldsymbol{\theta}} \xrightarrow{P} \underline{\boldsymbol{\theta}}$), we have

$$\begin{aligned}
\frac{1}{m} \sum_{i=1}^m \mathbf{Q}_i^{o\top} \hat{\boldsymbol{\Sigma}}_i^{-1} \mathbf{Q}_i^o &= \hat{\sigma}^{-2} \frac{m-p-2}{m^2} \sum_{i=1}^m \left(\mathbf{Q}_i^{o\top} \mathbf{Q}_i^o - \frac{\hat{\tau}^2}{\hat{\sigma}^2 + N_i \hat{\tau}^2} \mathbf{Q}_i^{o\top} \mathbf{1}_{N_i} \mathbf{1}_{N_i}^\top \mathbf{Q}_i^o \right) \\
&= \hat{\sigma}^{-2} \frac{m-p-2}{m^2} \sum_{i=1}^m \left(\mathbf{Q}_i^{o\top} \mathbf{Q}_i^o - \frac{\underline{\tau}^2}{\underline{\sigma}^2 + N_i \underline{\tau}^2} \mathbf{Q}_i^{o\top} \mathbf{1}_{N_i} \mathbf{1}_{N_i}^\top \mathbf{Q}_i^o \right) + \mathbf{r} \\
&= \frac{\underline{\sigma}^2}{\hat{\sigma}^2} \frac{m-p-2}{m^2} \sum_{i=1}^m \mathbf{Q}_i^\top \mathbf{D}_{M_i} \left(\frac{1}{\underline{\sigma}^2} \mathbf{I}_{N_i} - \frac{\underline{\tau}^2}{\underline{\sigma}^2(\underline{\sigma}^2 + N_i \underline{\tau}^2)} \mathbf{1}_{N_i} \mathbf{1}_{N_i}^\top \right) \mathbf{D}_{M_i}^\top \mathbf{Q}_i + \mathbf{r} \\
&= \frac{\underline{\sigma}^2}{\hat{\sigma}^2} \frac{m-p-2}{m^2} \sum_{i=1}^m \mathbf{Q}_i^\top \mathbf{V}_i^{-1} \mathbf{Q}_i + \mathbf{r} \\
&= (1 + o_p(1))(E[\mathbf{Q}^\top \mathbf{V} \mathbf{Q}] + o_p(1)) + \mathbf{r}
\end{aligned}$$

where

$$\mathbf{r} = \hat{\sigma}^{-2} \frac{m-p-2}{m^2} \sum_{i=1}^m \frac{\hat{\sigma}^2 \underline{\tau}^2 - \underline{\sigma}^2 \hat{\tau}^2}{(\hat{\sigma}^2 + N_i \hat{\tau}^2)(\underline{\sigma}^2 + N_i \underline{\tau}^2)} \mathbf{Q}_i^{o\top} \mathbf{1}_{N_i} \mathbf{1}_{N_i}^\top \mathbf{Q}_i^o.$$

If we can show that $\mathbf{r} = o_p(1)$, then by the Continuous Mapping Theorem, we get $m\widehat{Var}(\hat{\boldsymbol{\beta}}) \xrightarrow{P} (E[\mathbf{Q}^\top \mathbf{V} \mathbf{Q}])^{-1}$. To show $\mathbf{r} = o_p(1)$, by Assumption 1, regularity condition (2) and the fact that $\underline{\sigma}^2 > 0$, we get

$$\begin{aligned}
\|\mathbf{r}\| &\leq \hat{\sigma}^{-2} \frac{m-p-2}{m^2} \sum_{i=1}^m \frac{|\hat{\sigma}^2 \underline{\tau}^2 - \underline{\sigma}^2 \hat{\tau}^2|}{(\hat{\sigma}^2 + N_i \hat{\tau}^2)(\underline{\sigma}^2 + N_i \underline{\tau}^2)} \|\mathbf{Q}_i^{o\top} \mathbf{1}_{N_i} \mathbf{1}_{N_i}^\top \mathbf{Q}_i^o\| \\
&\leq \frac{|\hat{\sigma}^2 \underline{\tau}^2 - \underline{\sigma}^2 \hat{\tau}^2|}{\hat{\sigma}^4 \underline{\sigma}^2} \frac{m-p-2}{m} \frac{1}{m} \sum_{i=1}^m \|\mathbf{1}_{N_i}^\top \mathbf{Q}_i^o\|^2 \\
&= \frac{o_p(1)}{(\underline{\sigma}^2 + o_p(1))^2 \underline{\sigma}^2} O_p(1) \\
&= o_p(1).
\end{aligned}$$

Hence $m\widehat{Var}(\hat{\Delta}) \xrightarrow{P} 1/\{\pi(1-\pi)\mathbf{1}_n^\top E[\mathbf{V}]\mathbf{1}_n\}$, which is the second-row second-column entry of $(E[\mathbf{Q}^\top \mathbf{V} \mathbf{Q}])^{-1}$. If $\pi = 0.5$, then $m\widehat{Var}(\hat{\Delta}) \xrightarrow{P} 4(\mathbf{1}_n^\top E[\mathbf{V}]\mathbf{1}_n)^{-1}$, which happens to be the true asymptotic variance of $\hat{\Delta}$ under arbitrary misspecification of the working ANCOVA model that generates $\hat{\Delta}$. \square

B.2 Proof of Theorem 2

We inherit the notation from the proof of Theorem 1. We have shown that

$$\mathbf{V} = \mathbf{D}_M \left(\frac{1}{\sigma^2} \mathbf{I}_N - \frac{\tau^2}{\sigma^2(\sigma^2 + N\tau^2)} \mathbf{1}_N \mathbf{1}_N^\top \right) \mathbf{D}_M^\top,$$

which implies $\mathbf{V}\mathbf{1}_n = \frac{1}{\sigma^2 + N\tau^2} \mathbf{M}$. When $N_i = \tilde{n}$ for all i , we have, by Equation (3),

$$\frac{1}{(\underline{\sigma}^2 + \tilde{n}\underline{\tau}^2)^2} E[\{\mathbf{M}^\top(\mathbf{Y} - \mathbf{Q}\underline{\beta})\}^2] = \frac{\tilde{n}}{\underline{\sigma}^2 + \tilde{n}\underline{\tau}^2},$$

which implies that, assuming $\pi = 0.5$,

$$\begin{aligned} v &= 4(\mathbf{1}_n^\top E[\mathbf{V}]\mathbf{1}_n)^{-1} \\ &= \frac{4}{\tilde{n}}(\underline{\sigma}^2 + \tilde{n}\underline{\tau}^2) \\ &= \frac{4}{\tilde{n}^2} E[\{\mathbf{M}^\top(\mathbf{Y} - \mathbf{Q}\underline{\beta})\}^2] \\ &= 4E[\{(\bar{Y}^o - E[Y]) - \Delta^*(A - \pi) - \underline{\beta}_X^\top(\bar{\mathbf{X}}^o - E[\mathbf{X}])\}^2] \\ &= 4 \left\{ \text{Var}(\bar{Y}^o - \Delta^*A) - 2\text{Cov}(\bar{Y}^o - \Delta^*A, \underline{\beta}_X^\top \bar{\mathbf{X}}^o) + \underline{\beta}_X^\top \text{Var}(\bar{\mathbf{X}}^o) \underline{\beta}_X \right\} \\ &= 4 \left\{ \text{Var}(\bar{Y}^o - \Delta^*A) - \mathbf{c}^\top \text{Var}(\bar{\mathbf{X}}^o) \mathbf{c} + (\mathbf{c} - \underline{\beta}_X)^\top \text{Var}(\bar{\mathbf{X}}^o) (\mathbf{c} - \underline{\beta}_X) \right\}, \end{aligned}$$

where $\bar{Y}^o = \tilde{n}^{-1} \sum_{j=1}^n M_{\bullet,j} Y_{\bullet,j}$, $\bar{\mathbf{X}}^o = \tilde{n}^{-1} \sum_{j=1}^n M_{\bullet,j} \mathbf{X}_{\bullet,j}$ and $\mathbf{c} = \text{Var}(\bar{\mathbf{X}}^o)^{-1} \text{Cov}(\bar{\mathbf{X}}^o, \bar{Y}^o - \Delta^*A) = \text{Var}(\bar{\mathbf{X}}^o)^{-1} \text{Cov}(\bar{\mathbf{X}}^o, \bar{Y}^o)$.

For the cluster-level ANCOVA estimator, by Assumption 1 and regularity conditions, Wang et al. (2019) showed that the asymptotic variance of $\hat{\Delta}^{(\text{cl})}$ is

$$v^{(\text{cl})} = 4\text{Var}(\bar{Y}^o - \Delta^*A - \underline{\alpha}_{\bar{\mathbf{X}}^o}^\top \bar{\mathbf{X}}^o),$$

where $\underline{\alpha}_{\bar{\mathbf{X}}^o} = \text{Var}(\bar{\mathbf{X}}^o)^{-1} \text{Cov}(\bar{Y}^o - \Delta^*A, \bar{\mathbf{X}}^o) = \mathbf{c}$. Hence

$$\begin{aligned} v^{(\text{cl})} &= 4 \left\{ \text{Var}(\bar{Y}^o - \Delta^*A) - \mathbf{c}^\top \text{Var}(\bar{\mathbf{X}}^o) \mathbf{c} \right\} \\ &= v - 4(\mathbf{c} - \underline{\beta}_X)^\top \text{Var}(\bar{\mathbf{X}}^o) (\mathbf{c} - \underline{\beta}_X) \\ &\leq v. \end{aligned}$$

We next examine when $v^{(\text{cl})} = v$. For the mixed-model ANCOVA estimator, we have $\underline{\boldsymbol{\beta}} = (E[\mathbf{Q}^\top \mathbf{V} \mathbf{Q}])^{-1} E[\mathbf{Q}^\top \mathbf{V} \mathbf{Y}]$. When $N_i = \tilde{n}$ for all i , we can compute

$$\begin{aligned} \underline{\boldsymbol{\beta}}_{\mathbf{X}} &= E [(\mathbf{X} - E[\mathbf{X}])^\top \mathbf{V} (\mathbf{X} - E[\mathbf{X}])]^{-1} E [(\mathbf{X} - E[\mathbf{X}])^\top \mathbf{V} (\mathbf{Y} - E[\mathbf{Y}])] \\ &= \left\{ \frac{\tilde{n}}{\underline{\sigma}^2} \text{Var}(\mathbf{X}) - \frac{\underline{\tau}^2}{\underline{\sigma}^2 + \tilde{n}\underline{\tau}^2} \text{Var}(\mathbf{X}^{o\top} \mathbf{1}_{\tilde{n}}) \right\}^{-1} \left\{ \frac{\tilde{n}}{\underline{\sigma}^2} \text{Cov}(\mathbf{X}, Y) - \frac{\underline{\tau}^2}{\underline{\sigma}^2 + \tilde{n}\underline{\tau}^2} \text{Cov}(\mathbf{X}^{o\top} \mathbf{1}_{\tilde{n}}, \mathbf{Y}^{o\top} \mathbf{1}_{\tilde{n}}) \right\} \\ &= \left\{ \frac{\tilde{n}}{\underline{\sigma}^2} \text{Var}(\mathbf{X}) - \frac{\tilde{n}^2 \underline{\tau}^2}{\underline{\sigma}^2 + \tilde{n}\underline{\tau}^2} \text{Var}(\overline{\mathbf{X}}^o) \right\}^{-1} \left\{ \frac{\tilde{n}}{\underline{\sigma}^2} \text{Cov}(\mathbf{X}, Y) - \frac{\tilde{n}^2 \underline{\tau}^2}{\underline{\sigma}^2 + \tilde{n}\underline{\tau}^2} \text{Cov}(\overline{\mathbf{X}}^o, \overline{Y}^o) \right\}. \end{aligned}$$

Since $\text{Var}(\overline{\mathbf{X}}^o)$ is positive definite, then

$$\begin{aligned} v^{(\text{cl})} = v &\Leftrightarrow (\mathbf{c} - \underline{\boldsymbol{\beta}}_{\mathbf{X}})^\top \text{Var}(\overline{\mathbf{X}}^o) (\mathbf{c} - \underline{\boldsymbol{\beta}}_{\mathbf{X}}) = 0 \\ &\Leftrightarrow \mathbf{c} - \underline{\boldsymbol{\beta}}_{\mathbf{X}} = \mathbf{0} \\ &\Leftrightarrow \left\{ \frac{\tilde{n}}{\underline{\sigma}^2} \text{Var}(\mathbf{X}) - \frac{\tilde{n}^2 \underline{\tau}^2}{\underline{\sigma}^2 + \tilde{n}\underline{\tau}^2} \text{Var}(\overline{\mathbf{X}}^o) \right\} \text{Var}(\overline{\mathbf{X}}^o)^{-1} \text{Cov}(\overline{\mathbf{X}}^o, \overline{Y}^o) \\ &\quad = \frac{\tilde{n}}{\underline{\sigma}^2} \text{Cov}(\mathbf{X}, Y) - \frac{\tilde{n}^2 \underline{\tau}^2}{\underline{\sigma}^2 + \tilde{n}\underline{\tau}^2} \text{Cov}(\overline{\mathbf{X}}^o, \overline{Y}^o) \\ &\Leftrightarrow \text{Var}(\overline{\mathbf{X}}^o)^{-1} \text{Cov}(\overline{\mathbf{X}}^o, \overline{Y}^o) = \text{Var}(\mathbf{X})^{-1} \text{Cov}(\mathbf{X}, Y), \end{aligned}$$

which completes the proof.

B.3 Proof of Theorem 3

By Assumption 1 (a-b), Assumption 2, and regularity conditions in the Appendix, Theorem 1 of Wang et al. (2021) implies the consistency and asymptotic normality of $\widehat{\Delta}$ under stratified randomization. Furthermore

$$\tilde{v} = v - \frac{1}{\pi(1-\pi)} E[E\{(A - \pi)IF(\mathbf{Y}, A, \mathbf{X}, \mathbf{M}; \underline{\boldsymbol{\theta}})|S\}^2],$$

where $IF(\mathbf{Y}, A, \mathbf{X}, \mathbf{M}; \underline{\boldsymbol{\theta}})$ is defined in Equation (2).

If S is adjusted in the mixed-model ANCOVA model, then $E[\boldsymbol{\psi}(\mathbf{Y}, A, \mathbf{X}, \mathbf{M}; \underline{\boldsymbol{\theta}})] = \mathbf{0}$ implies that

$$E[\mathbf{1}_n^\top \mathbf{V} (\mathbf{Y} - \mathbf{Q}\underline{\boldsymbol{\beta}})|S] = 0.$$

Hence

$$\begin{aligned}\tilde{v} &= v - \frac{1}{\pi(1-\pi)} E \left[E \left\{ \frac{(A-\pi)^2}{\pi(1-\pi) \mathbf{1}_n^\top E[\mathbf{V}] \mathbf{1}_n} \mathbf{1}_n^\top \mathbf{V} (\mathbf{Y} - \mathbf{Q}\underline{\boldsymbol{\beta}}) \middle| S \right\}^2 \right] \\ &= v - \frac{(1-2\pi)^2}{\pi^3(1-\pi)^3 (\mathbf{1}_n^\top E[\mathbf{V}] \mathbf{1}_n)^2} E[E\{A \mathbf{1}_n^\top \mathbf{V} (\mathbf{Y} - \mathbf{Q}\underline{\boldsymbol{\beta}}) | S\}^2],\end{aligned}$$

which implies $\tilde{v} = v$ if $\pi = 0.5$.

B.4 Results for the mixed-model ANCOVA model that correctly specifies the mean and variance structure

Suppose that $E[\mathbf{Y}_i | A_i, \mathbf{X}_i] = \beta_0 \mathbf{1}_n + A_i \beta_A \mathbf{1}_n + \boldsymbol{\beta}_X^\top \mathbf{X}_i$ and $Var(\mathbf{Y}_i - E[\mathbf{Y}_i | A_i, \mathbf{X}_i]) = \sigma^2 \mathbf{I}_n + \tau^2 \mathbf{1}_n \mathbf{1}_n^\top$ for some $\boldsymbol{\theta}^* = (\beta_0, \beta_A, \boldsymbol{\beta}_X, \sigma^2, \tau^2) \in \Theta$. Then it is straightforward to show that $\underline{\boldsymbol{\theta}} = \boldsymbol{\theta}^*$. Then the asymptotic variance of $\hat{\Delta}$ is $v = 4\{E[\frac{N}{\sigma^2 + N\tau^2}]\}^{-1}$. By Jensen's inequality, we have

$$v \geq 4 \left\{ \frac{E[N]}{\sigma^2 + E[N]\tau^2} \right\}^{-1},$$

which indicates that the variation of cluster sizes will result in precision loss compared to constant cluster sizes.

For comparison between v and $v^{(cl)}$, we have $\underline{\boldsymbol{\alpha}}_{\bar{\mathbf{X}}^o} = \boldsymbol{\beta}_X$ and

$$\begin{aligned}v^{(cl)} &= 4Var(\bar{Y}^o - \Delta^* A - \underline{\boldsymbol{\alpha}}_{\bar{\mathbf{X}}^o}^\top \bar{\mathbf{X}}^o) \\ &= 4Var\left(\frac{1}{N} \mathbf{M}^\top \{\mathbf{Y} - E[\mathbf{Y} | A, \mathbf{X}]\}\right) \\ &= 4E\left[Var\left(\frac{1}{N} \mathbf{M}^\top \{\mathbf{Y} - E[\mathbf{Y} | A, \mathbf{X}]\} \middle| N\right)\right] + 4Var\left(E\left[\frac{1}{N} \mathbf{M}^\top \{\mathbf{Y} - E[\mathbf{Y} | A, \mathbf{X}]\} \middle| N\right]\right) \\ &= 4E\left[\frac{\sigma^2 + N\tau^2}{N}\right].\end{aligned}$$

Hence by the Hölder's inequality, we have

$$\frac{v^{(cl)}}{v} = E\left[\frac{\sigma^2 + N\tau^2}{N}\right] E\left[\frac{N}{\sigma^2 + N\tau^2}\right] \geq 1,$$

which indicates that $v^{(cl)} \geq v$. In the special case that N is fixed, then $v^{(cl)} = v$.

C Empirical comparison of REML and ML

As discussed in Section 6 of the main manuscript, here we provide additional numerical results (in the following Table 1 and Table 2) to compare the maximum restricted maximum likelihood (REML) and maximum likelihood (ML) estimation for the mixed-model ANCOVA parameters; the former is known to reduce the bias of the variance component estimators, while the latter is a more standard approach which we consider to prove our main results. The purpose of this additional comparison is provide some preliminary evidence that the our theoretical results may also hold for REML-based mixed-model ANCOVA analysis of CRTs. A formal proof of the robustness of the REML estimator under arbitrary model misspecification is subject to additional research.

References

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- Wang, B., Susukida, R., Mojtabai, R., Amin-Esmaeili, M., and Rosenblum, M. (2021). Model-robust inference for clinical trials that improve precision by stratified randomization and covariate adjustment. *Journal of the American Statistical Association*.

Table 1: Simulation results for Scenarios 1–3 comparing ML and REML estimation in mixed models. The performance metrics are bias, empirical standard error (Emp SE), averaged model-based standard error (ASE), coverage probability of nominal 0.95 confidence intervals based on normal approximation and model-based standard error (CP), and relative efficiency to the mixed-model unadjusted ML estimator (RE).

	Mixed models	Method	Bias	Emp SE	ASE	CP	RE	
Scenario 1	$m = 20$	unadjusted	ML	0.00	0.95	1.00	0.94	1.00
			REML	0.00	0.96	0.98	0.95	1.00
		ANCOVA	ML	-0.01	0.97	1.01	0.93	0.96
			REML	0.00	0.97	0.98	0.94	0.96
	$m = 200$	unadjusted	ML	0.01	0.29	0.30	0.95	1.00
			REML	0.01	0.29	0.30	0.95	1.00
		ANCOVA	ML	0.01	0.30	0.30	0.95	0.96
			REML	0.01	0.30	0.30	0.95	0.96
Scenario 2	$m = 20$	unadjusted	ML	-0.02	1.29	1.29	0.93	1.00
			REML	-0.02	1.29	1.28	0.94	1.00
		ANCOVA	ML	0.00	1.09	1.09	0.92	1.40
			REML	0.00	1.09	1.08	0.94	1.40
	$m = 200$	unadjusted	ML	-0.01	0.41	0.41	0.95	1.00
			REML	-0.01	0.41	0.41	0.950	1.00
		ANCOVA	ML	0.00	0.34	0.34	0.95	1.40
			REML	0.00	0.34	0.34	0.95	1.40
Scenario 3	$m = 20$	unadjusted	ML	-0.01	1.00	1.02	0.94	1.00
			REML	-0.01	1.00	1.01	0.95	1.00
		ANCOVA	ML	-0.01	0.95	0.99	0.94	1.11
			REML	-0.01	0.95	0.95	0.94	1.11
	$m = 200$	unadjusted	ML	0.01	0.31	0.31	0.95	1.00
			REML	0.01	0.31	0.31	0.95	1.00
		ANCOVA	ML	0.01	0.29	0.29	0.95	1.12
			REML	0.01	0.29	0.29	0.95	1.12

Table 2: Summary of data analyses results comparing ML and REML estimation in mixed models: point estimate of the average treatment effect (Est), model-based estimator for standard error (SE), 95% confidence interval (CI), and proportion variance reduction compared to the unadjusted ML estimator (PVR). Positive (negative) PVR indicates that covariate adjustment leads to variance reduction (inflation).

Study name	Estimators	method	Est	SE	95% CI	PVR
TSSSH	mixed-model unadjusted	ML	-1.29	2.08	(-5.36, 2.78)	-
		REML	-1.29	2.08	(-5.36, 2.78)	0%
	mixed-model ANCOVA	ML	-2.22	2.09	(-6.32, 1.87)	-1%
		REML	-2.22	1.98	(-6.11, 1.67)	9%
IECDZ	mixed-model unadjusted	ML	0.08	0.14	(-0.19, 0.35)	-
		REML	0.08	0.14	(-0.19, 0.35)	0%
	mixed-model ANCOVA	ML	0.08	0.15	(-0.22, 0.37)	-21%
		REML	0.08	0.14	(-0.20, 0.36)	-9%
WFHS	mixed-model unadjusted	ML	0.16	0.07	(0.01, 0.30)	-
		REML	0.16	0.07	(0.01, 0.30)	-1%
	mixed-model ANCOVA	ML	0.21	0.05	(0.12, 0.31)	56%
		REML	0.21	0.05	(0.11, 0.31)	55%