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ROBUST PROCEDURES FOR BIOASSAYS AND BIOEQUIVALENCE STUDIES

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SUMMARY. In bioassays, inference on relative potency and bioequivalence (of a new preparation relative to a standard one) constitute the main theme of studies. A conditional quantile process based on the k-nearest neighborhood method is proposed for stochastic dose designs. Minimum chi-square type estimators are considered and incorporated in the test for the fundamental assumption of the assay. Bias correction by jackknife methods is discussed. As an illustration, ARIC ultrasound data are analyzed.

1. Introduction

In an indirect quantitative bioassay, a new (test) preparation (T) and an old (standard) one (S), not necessarily of the same (bio)chemical constitution, are compared by means of the reactions that follow their applications to some biological organisms. In conventional statistical modeling and analysis, it is assumed that responsewise the test preparation behaves as if it is a dilution (or concentration) of the standard one. It is of interest to assess the relative potency of the test preparation with respect to the standard one, interpreted in the above mode. Indirect bioassays relate to dose-response regression model with some built-in structures; in the dilution model, corresponding a dose x, the response Y_T and Y_S for the test and standard preparation are assumed to satisfy the following:

$$E(Y_T|x) = h_T(x) = h_S(\rho x), \text{ where } h_S(x) = E(Y_S|x), \tag{1.1}$$

for all x and for some $\rho > 0$, where ρ stands for the relative potency of the test preparation with respect to the standard one. In parametric models for indirect quantitative bioassays it is typically assumed that the tolerance distribution is (log-) normal or (log-)logistic. However, as the dose and response variables are generally nonnegative, and the tolerance distribution is usually positively skewed, to achieve symmetry (a prerequisite for normal/logistic distributions), one typically chooses a

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log metameter (transformation). Then the dose-response regression relationship is given by

$$E(Y_T|x) = h_T(x) = h_S(\mu + x) = E(Y_S|x + \mu), \text{ for all } x \text{ and for some } \mu \in R, \quad (1.2)$$

where $\mu = \log(\rho)$. It may be noted that a log-metameter does not necessarily induce normality or logistic form for the transformed tolerance distribution, and therefore robustness considerations are of importance in practice. Note that (1.2) constitutes the *fundamental assumption* of an assay. The main interest is to estimate the relative potency ρ and to test for the validity of the fundamental assumption.

Parametric statistical procedures for these problems, discussed in detail in Finney (1964), are usually based on the assumption that the distribution of Y is normal, lognormal, logistic or loglogistic. Based on (generalized) linear models, the usual *maximum likelihood estimator* (MLE) is consistent and asymptotically normally distributed when the assumed model holds. However, these parametric estimates are generally not very robust against departures from model assumptions. Rank based estimates of relative potency have been considered by Sen (1963, 1968, 1971), Shorack (1966) and Rao and Little (1976), among others. These estimates are invariant under the choice of any monotone transformation on the dose, and, besides being robust, are generally quite efficient for normal, log-normal, logistic or other common forms of the tolerance distributions.

In a classical indirect quantitative bioassay, we have a set of distinct dose levels and responses of different subjects for each dose level. In this set-up, we have independent samples at each dose level and for each preparation. Under normality of the errors, the mean responses at these dose levels lead to the MLE of the parameters in (1.2) where additionally linear regression forms are assumed. The MLE are in general not so robust to possible model departures, and hence, we like to incorporate alternative approaches that have better robustness prospects. Whenever we have linear dose-response regression functions, it may be possible to use *regression quantiles*, proposed by Koenker and Bassett (1978), which have good robustness properties, though computationally may not be that convenient. In simple designs, as we shall consider here, subsample quantiles might work out more conveniently. For each dose we may use a specified sample quantile (or median) instead of the mean, and that way induce more robustness properties. In Section 2, we elaborate this approach for fixed-dose designs.

The situation is relatively more complex with stochastic dose designs. We borrow the idea of conditional quantiles in nonparametric regression, and extend the findings to stochastic dose-designs in bioassay problems. Bhattacharya and Gangopadhyay (1990) proposed $k-$nearest neighborhood ($k-$NN) estimator of a conditional quantile, and the corresponding confidence interval problem was studied by Gangopadhyay and Sen (1990). We incorporate the conditional quantiles of the response variable in the regression relation in (1.2), and test for the validity of the fundamental assumption, as well as, estimate the relative potency. In section 3, we introduce the conditional quantile process, and illustrate their role in indirect quantitative bioassays. Bias correction procedures based on the jackknife methods are

also discussed there. The concluding section deals with some numerical examples and simulation results.

2. Fixed Dose Design: Robustness Perspectives

In this section we consider the case that the dose level for both the test and the standard preparations are fixed (nonstochastic). For simplicity, we consider a symmetric, balanced $2m$-point design, and if necessary, by location-scale transformation, we choose congruent dose-levels for both the preparations. Let $(x_1, \cdots, x_m)$ be the dose levels for each of the two preparations and n_0 be the number of responses at each dose level. Thus, we have the following two sets of responses:

	Standard Preparation				Test preparation			
Dosage	x_1	x_2	$\cdots$	x_m	x_1	x_2	$\cdots$	x_m
	Y^S_{11}	Y^S_{21}	$\cdots$	Y^S_{m1}	Y^T_{11}	Y^T_{21}	$\cdots$	Y^T_{m1}
	$\cdots$	$\cdots$	$\cdots$	$\cdots$	$\cdots$	$\cdots$	$\cdots$	$\cdots$
	$Y^S_{1n_0}$	$Y^S_{2n_0}$	$\cdots$	$Y^S_{mn_0}$	$Y^T_{1n_0}$	$Y^T_{2n_0}$	$\cdots$	$Y^T_{mn_0}$

Conventionally, a linear dosage (=logdose) - response relationship is taken for granted, though we like to be less specific about the form of the tolerance distributions. Keeping this in mind, in this section, we assume the following relationship:

$$\xi^T_j = \alpha_T + \beta_T x_j \text{ and } \xi^S_j = \alpha_S + \beta_S x_j \text{ for } x_j \in \{x_1, \cdots, x_m\}, \tag{2.2}$$

where ξ^i_j is p-th quantile of the response at the dose x_j for preparation i $(i = T, S)$. Then using (1.2) and (2.1), we reformulate the fundamental assumption as:

$$\beta_T = \beta_S = \beta; \quad \alpha_T - \alpha_S = \beta\mu; \ \mu = \log \rho. \tag{2.2}$$

Thus, testing for the validity of the fundamental assumption is isomorphic to testing the parallelism of the two dose-regression lines (without possibly imposing normality or some other specific distributional property on the error components). For this, we proceed as in Sen (1971): Let

$$W^i_{(lj,rs)} = (Y^i_{ls} - Y^i_{jr})/(x_l - x_j),\ r, s = 1, \cdots, n_0,\ 1 \leq < l \leq m,\ i = S, T,$$

be the divided differences for each preparation. The median of these $m(m-1)n_0^2$ entities is $\hat{\beta}$, the pooled estimator of the hypothesized common β (Sen, 1971). Asymptotic normality of $\sqrt{mn_0}(\hat{\beta} - \beta)$ under the parallelism of the two lines has also been considered there. Let us consider then the residuals

$$\hat{Y}^i_{jr} = Y^i_{jr} - \hat{\beta} x_j,\ r = 1, \ldots, n_0, j = 1, \ldots, m; i = S, T.$$

Further, let $\hat{U}_T$ and $\hat{U}_S$ be respectively the Kendall tau statistic based on the residuals in the test and standard preparations. Also, let V^2_n be the null hypothesis

variance of Kendall tau statistic (same for both the preparation under the design considered here). Sen (1971) proposed the test statistic

$$Z = \{\hat{U}_T^2 + \hat{U}_S^2\}/V_n^2, \tag{2.3}$$

and showed that under the null hypothesis of parallelism of the two regression lines, Z has approximately the chi square distribution with 1 degree of freedom (DF). Thus, the critical values can be approximated by the corresponding percentile points of the chi square distribution with 1 DF. For drawing inference on μ, we use the following asymptotic normality results: as $n \to \infty$,

$$\begin{aligned} n_0^{1/2}(\hat{\xi}_i^T - \xi_i^T) &\xrightarrow{\mathcal{D}} N\left(0, pq/f_i^2(\xi_i^T)\right) \\ n_0^{1/2}(\hat{\xi}_i^S - \xi_i^S) &\xrightarrow{\mathcal{D}} N\left(0, pq/f_i^2(\xi_i^S)\right) \end{aligned} \tag{2.4}$$

where $\hat{\xi}_i^j$ is the p-sample quantile at dose level i for treatment j and $f_i(\cdot)$ is the error density of response at x_i (assumed to be the same for both treatments). If we assume that the error density remains the same at each dose level and for each preparation, then we minimize the following quadratic with respect to μ (at the estimated value $\hat{\beta}$):

$$\begin{aligned} Q(\mu) &= \textstyle\sum_{i=1}^m n_0 \left((\hat{\xi}_i^T - \xi_i^T) - (\hat{\xi}_i^S - \xi_i^S)\right)^2 \\ &= \textstyle\sum_{i=1}^m n_0(\hat{\xi}_i^T - \hat{\xi}_i^S - \beta\mu)^2 \end{aligned} \tag{2.5}$$

and obtain a least square type estimator of μ. Thus, we obtain

$$\hat{\mu} = \sum_{i=1}^m (\hat{\xi}_i^T - \hat{\xi}_i^S)/m\hat{\beta}. \tag{2.6}$$

By virtue of (2.4), (2.6), asymptotic normality of $\sqrt{n_0}(\hat{\beta} - \beta)$ and the Slutzky theorem, the asymptotic normality of $\sqrt{n_0}(\hat{\mu} - \mu)$ follows by standard steps. This justifies the adoption of common resampling methods such as the jackknife or the bootstrap to construct confidence intervals for μ or equivalently for ρ. The proposed method is more robust than the MLE/LSE methods based on assumed normality of the response. If we allow different numbers of data points, i.e., let $(n_1^i, \cdots, n_m^i)$ be the number of observed responses at $(x_1, \cdots, x_m)$ for the test and standard preparation ($i = T, S$, respectively). Then (2.6) becomes

$$\hat{\mu} = \frac{\sum_{i=1}^m \frac{n_i^T n_i^S}{n_i^T + n_i^S}(\hat{\xi}_i^T - \hat{\xi}_i^S)}{\sum_{i=1}^m \frac{n_i^T n_i^S}{n_i^T + n_i^S}\hat{\beta}}.$$

The asymptotic normality results also hold for estimators of μ, β in this case also, and as a result resampling methods can be used to attach a confidence interval for ρ.

3. Stochastic Doses and Conditional Quantiles

We motivate our procedure with an outline of conditional quantile processes. Let $\{(X_i, Y_i), i \geq 1\}$ be a sequence of i.i.d. random vectors with a distribution function $\Pi(x, y), (x, y) \in R^2$. Let $F(x) = \Pi(x, \infty)$, $x \in R$ and let $G(y|x)$ be the conditional d.f. of Y given $X = x$, for $y \in R$, $x \in R$. A conditional quantile function (of Y given $X = x$) is defined by

$$\xi_p(x) = \inf\{y : G(y|x) \geq p\},\ x \in R,\ (0 < p < 1).$$

Consider the transformation $(X_i, Y_i) \to (Z_i, Y_i)$ where $Z_i = |X_i - x|$. For the collection $\{(Z_1, Y_1), \cdots, (Z_n, Y_n)\}$ of r.v.'s, let $Z_{n1} < \cdots < Z_{nn}$ be the order statistics corresponding to $Z_1, \cdots, Z_n$, and let $Y_{n1}, \cdots, Y_{nn}$ be the induced (or concomitants of) order statistics. (i.e., $Y_{ni} = Y_j$ if $Z_{ni} = Z_j$, for $i, j = 1, \cdots, n$). For every positive integer $k(\leq n)$, the k-NN empirical d.f. of Y (with respect to x) is given by

$$\hat{G}_{nk}(y) = k^{-1} \sum_{i=1}^{k} 1(Y_{ni} \leq y),\ y \in R,$$

where $1(A)$ stands for the indicator function of the set A. The k-NN estimator of ξ, due to Bhattacharya and Gangopadhyay (1990), is defined by

$$\begin{aligned} \hat{\xi}_{nk} &= \text{ the } [kp]\text{-th order statistic of } Y_{n1}, \cdots, Y_{nk} \\ &= \inf\{y : \hat{G}_{nk}(y) \geq k^{-1}\,[kp]\}. \end{aligned}$$

For the asymptotic normality results, one needs to choose an increasing sequence $\{k_n\}$, in such a way that $n^{-1}k_n \to 0$, as $n \to \infty$; usually, we take $k_n = O(n^{\lambda})$, for some $\lambda \in (0, 1)$. To balance the asymptotic bias and mean square errors, Bhattacharya and Gangopadhyay (1990) chose $\lambda = 4/5$, and established the asymptotic normality of the k-NN estimator: for every $t \in [a, b]$ as $n \to \infty$

$$n^{2/5}[\hat{\xi}_{n,[n^{4/5}t]} - \xi] \xrightarrow{\mathcal{D}} N(\beta t^2, p(1-p)t^{-1}/g(\xi)^2),$$

where $g(\cdot) = g(\cdot|x)$ is the conditional density of Y given $X = x$ and

$$\beta(\xi) = -\frac{1}{23f^3(x)g(\xi)} \cdot [f(x)G_{xx}(\xi|x)\ + 2f'(x)G_x(\xi|x)]$$

and

$$G_x(y|x) = (\partial/\partial x)G(y|x),\ G_{xx}(y|x) = (\partial^2/\partial x^2)G(y|x).$$

Gangopadhyay and Sen (1990) showed the bias term (βt^2) can be eliminated when $k_n = O(n^{4/5-2\delta}),\ 0 < \delta < 1/10$. They showed that for every $k : k = [tn^{4/5-2\delta}]$, $t \in [a, b]$, as $n \to \infty$

$$n^{2/5-\delta}[\hat{\xi}_{nk} - \xi] \xrightarrow{\mathcal{D}} N(0, t^{-1}p(1-p)/g^2(\xi)).$$

They also formulated the construction of (local) bootstrap confidence intervals for ξ based on the k-NN methods and asymptotic normality of the bootstrap estimator.

Let $\xi(x)$ be the p-quantile of the d.f. $G(\cdot|x),\ x \in [x_l, x_u] = \mathcal{X} \subset \mathbf{R}$, and let $\hat{\xi}_{n,k_n}(x)$ be the k-NN estimator for $k_n = O(n^{4/5-2\delta})$ where Z_{ni} are defined with respect to the pivot x. Note that these Z_{ni} are the induced order statistics corresponding to the nearest neighbors of x (i.e. those X_i's which are closest to x). As such, if we consider two distinct points, say x_1 and x_2, then the two sets of X_i forming the corresponding nearest neighbors would have at most k_n members in common. The proportion of the two subsets' common elements will be asymptotically $o(n^{-1}k_n)$, and we may immediately say that $\hat{\xi}_{n,k_n}(x_1)$ and $\hat{\xi}_{n,k_n}(x_2)$ are asymptotically independent. We set $W_n = \{W_n(x), x \in \mathcal{X}\}$ by letting

$$W_n(x) = k_n^{1/2}\left[\hat{\xi}_{n,k_n}(x) - \xi(x)\right],\ \ x \in \mathcal{X},$$

where $\mathcal{X} \subset R^1$ is a compact interval. Then the asymptotic behavior of W_n is depicted entirely by the pointwise asymptotic normality and the stochastic dependence pattern only in a shrinking neighborhood of fixed points. This we present below.

From the pointwise asymptotic normality result discussed above, we have, as $n \to \infty$,

$$W_n(x) \xrightarrow{\mathcal{D}} W(x),\ \text{for every } x \in \mathcal{X},$$

where $W(x)$ is normal with mean 0 and variance $pq/(g^2(\xi(x)|x))$ for $k_n = tn^{4/5-2\delta}$. For distinct points (x), $W_n(x)$ are asymptotically stochastically independent. However, in a shrinking neighborhood of a fixed point, they are not. Gangopadhyay and Sen (1993) defined shrinking neighborhood by the sets of x relative to a given x_0 as

$$I_n(x_0, K) = \{x : |x - x_0| \le Kn^{-1}k_n\};\ k_n = O(n^{4/5-2\delta}),$$

where K is a finite positive number. They considered a stochastic process $W_n^0 = \{W_n^0(t), t \in [t_0, t_1]\}$ where

$$W_n^0(t) = W_n(x_0 + n^{-1}k_n t),\ t \in [t_0, t_1]. \tag{3.1}$$

Under the assumption of a finite fourth moment of $Z_{ni}^* = G^{-1} \circ G(Z_{ni}|Y_{ni})$ for $i = 1, \cdots, n$, they proved that (3.1) is tight, where $t \in C = [-K, K]$ such that $Kf(x_0) \le 1$, This, in turn implies the weak convergence of W_n^0 to W^0 where W^0 is Gaussian.

In the current context, we have the two independent stochastic processes corresponding to the two preparations (T and S). Denoting them by $W_n^T(x)$ and $W_n^S(x)$ respectively, we have from the above asymptotic results,

$$W_n^T(x) = k_n^{1/2}[\hat{\xi}_{n,k_n}^T(x) - \xi_T(x)] \xrightarrow{\mathcal{D}} W_T(x), \text{ for every } x \in \mathcal{X} \text{ as } n \to \infty, \tag{3.2}$$

$$W_n^S(x) = k_n^{1/2}[\hat{\xi}_{n,k_n}^S(x) - \xi_S(x)] \xrightarrow{\mathcal{D}} W_S(x), \text{ for every } x \in \mathcal{X} \text{ as } n \to \infty, \tag{3.3}$$

where W_T and W_S are independent Gaussian processes for the test and the standard preparations. Under the fundamental assumption,

$$\xi_T(x) = \xi_S(x+\mu) \text{ for every } x \in \mathcal{X} \text{ and for some } \mu, \tag{3.4}$$

we have from (3.2)-(3.3),

$$\begin{aligned} W_n^T(x) - W_n^S(x+\mu) &= k_n^{1/2}\left\{\hat{\xi}_{n,k_n}^T(x) - \hat{\xi}_{n,k_n}^S(x+\mu)\right\} \\ &\xrightarrow{\mathcal{D}} W_T(x) - W_S(x+\mu), \text{ as } n \to \infty \text{ for any given } x \in \mathcal{X}, \end{aligned} \tag{3.5}$$

where

$$W_T(x) \sim N\left(0, \frac{pq}{g_T^2(\xi_T(x))}\right), \tag{3.6}$$

$$W_S(x+\mu) \sim N\left(0, \frac{pq}{g_S^2(\xi_S(x+\mu))}\right), \tag{3.7}$$

and where $g_T(\cdot)$ and $g_S(\cdot)$ are the conditional densities of the test and standard preparations. Again, under (3.4), we may assume that $g_T(\xi_T(x)) = g_S(\xi_S(x+\mu))$, $\forall x$. Thus, under the fundamental assumption (3.4), we have a common asymptotic variance in (3.6) and (3.7), and we denote this by σ_x^2. We have already noticed that for distinct points the expressions on left hand side of (3.5) are asymptotically independent, while they have local dependence in a shrinking neighborhood of such points. In practice, we therefore take recourse to a set of discrete points and make use of the asymptotic independence of the quantities on which our test and estimates are to be based.

We let $\boldsymbol{x} = (x_1, \cdots, x_m)$ be m points of dose levels selected from the region of interest $\mathcal{X}$ and we consider the following quadratic function

$$Q_m(\mu) = k_n \sum_{i=1}^{m} (\hat{\xi}_{n,k_n}^T(x_i) - \hat{\xi}_{n,k_n}^S(x_i+\mu))^2/(2\sigma_{x_i}^2) \tag{3.8}$$

where $\sigma_{x_i}^2$ is the variance of $W_T(x_i)$ and $W_S(x_i+\mu)$ in (3.6) and (3.7) for any given $x_i \in \mathcal{X}$. Then $Q_m(\mu)$ is the sum of squares of asymptotically i.i.d. normals so it has asymptotically the central chi-square distribution with m degree of freedom. We also denote by

$$b_i(\mu) = (\partial/\partial\mu)\xi_S(x_i+\mu), \ i = 1, \ldots, m,$$

and pretend, for the time being, that the $b_i(\mu)$, σ_{x_i} are known. Then in a neighborhood of $x_i + \mu$, we may write

$$\begin{aligned} &\hat{\xi}_S(x_i+\mu+t) - \hat{\xi}_S(x_i+\mu) \\ &\quad = \xi_S(x+\mu+t) - \xi_S(x_i+\mu) + k_n^{-1/2}[W_N^S(x_i+\mu+t) - W_n^S(x_i+\mu)] \\ &\quad = tb_i(\mu+ht) + o_p(k_n^{-1/2}) \ (0 < h < 1). \end{aligned} \tag{3.9}$$

This provides a locally linear approximation for $\hat{\xi}_S(x_i + \mu + t)$ whenever $t = O_p(k_n^{-1/2})$. Thus, we minimize (3.8) with respect to μ and obtain the estimating equation:

$$\frac{\partial}{\partial \mu} Q_m(\mu)\Big|_{\mu=\hat{\mu}_{mn}} = -k_n \sum_{i=1}^{m} \left(\hat{\xi}^T_{n,k_n}(x_i) - \hat{\xi}^S_{n,k_n}(x_i + \hat{\mu}_{mn})\right) \frac{b_i(\hat{\mu}_{mn})}{2\sigma^2_{x_i}} = 0, \quad (3.10)$$

where $\hat{\mu}_{mn}$ refers to the solution. Further, writing $b_i = b_i(\mu)$, we have from the above

$$\sum_{i=1}^{m} \left(\hat{\xi}^T_{n,k_n}(x_i) - \hat{\xi}^S_{n,k_n}(x_i + \mu) + (\mu - \hat{\mu}_{mn})b_i + o(||\mu - \hat{\mu}_{mn}||)\right)(b_i/2\sigma^2_{x_i}) = 0.$$

So, we have the following asymptotic normality result for $\hat{\mu}_{mn}$.

$$\begin{aligned} & k_n^{1/2}(\hat{\mu}_{mn} - \mu) \cdot \sqrt{\sum (b_i^2/\sigma^2_{x_i})} \\ &= \sum_{i=1}^{m} k_n^{1/2}(\hat{\xi}^T_{n,k_n}(x_i) - \hat{\xi}^S_{n,k_n}(x_i + \mu))(b_i/2\sigma^2_{x_i})/\sqrt{\sum b_i^2\sigma^2_{x_i}} + o(||\mu - \hat{\mu}_{mn}||)k_n^{1/2} \\ &\xrightarrow{\mathcal{D}} N(0,1) \text{ as } n \to \infty. \end{aligned} \quad (3.11)$$

From (3.8), we have

$$\begin{aligned} Q_m(\mu) &= Q_m(\hat{\mu}_{mn}) + (\mu - \hat{\mu}_{mn})Q_m^{(1)}(\mu)\Big|_{\mu=\hat{\mu}_{mn}} \\ &\quad + (\mu - \hat{\mu}_{mn})^2/2 \cdot Q_m^{(2)}(\mu)\Big|_{\mu=\hat{\mu}_{mn}} + o(||\mu - \hat{\mu}_{mn}||^2), \end{aligned} \quad (3.12)$$

where $Q_m^{(1)}$ and $Q_m^{(2)}$ are the first and second derivatives of $Q_m(\mu)$ with respect to μ. Finally, we have $Q_m^{(1)}(\mu)\Big|_{\mu=\hat{\mu}_{mn}} = 0$ and

$$\frac{(\mu - \hat{\mu}_{mn})^2}{2} Q_m^{(2)}(\mu)\Big|_{\mu=\hat{\mu}_{mn}} = \frac{(\mu - \hat{\mu}_{mn})^2}{2} k_n \sum (b_i^2/2\sigma^2_{x_i}) \to \chi^2_1 \text{ as } n \to \infty. \quad (3.13)$$

From (3.8) and (3.13) we obtain that when the fundamental assumption holds, $Q_m(\hat{\mu}_{mn}) \to \chi^2_{m-1}$ as $n \to \infty$. However, in the above expression (as well as for the one for $\hat{\mu}_{mn}$), we tacitly assume that the $\sigma_{x_i}, b_i(\mu)$ are all known. What we could do is to estimate the σ_{x_i} by the classical bootstrap method or jackknifing (as in Gangopadhyay and Sen 1990, 1993), and we may also exploit the local linearity in (3.9) to estimate the $b_i(\mu)$ by the same resampling methods. As such, we define $\hat{\mu}^*_{mn}$ as the solution for (3.10) when we use these estimators of the weighing factors b_i/σ_{x_i}, and let

$$Q_m(\hat{\mu}^*_{mn}) = Q^*_{mn} = k_n \sum_{i=1}^{m} (\hat{\xi}^T_{n,k_n}(x_i) - \hat{\xi}^S_{n,k_n}(x_i + \hat{\mu}^*_{mn}))^2 \tilde{\sigma}^{-1}_{ii}, \quad (3.14)$$

where $\tilde{\sigma}_{ii}$ is the bootstrap estimator of $Var(\hat{\xi}^T_{n,k_n}(x_i) - \hat{\xi}^S_{n,k_n}(x_i + \hat{\mu}_{mn}))$. Using the Slutzky theorem, it can be shown that when the fundamental assumption holds, Q^*_{mn} has asymptotically the central chi square distribution with $m-1$ degrees of freedom. Thus, we consider a test for the validity of the fundamental assumption wherein we reject the null hypothesis (of parallelism in (3.4)) when Q^*_{mn} is large (against the significance level that can be obtained from the central chi square distribution with $m-1$ degrees of freedom. We may note in the passing that the computation of the estimator $\hat{\mu}^*_{mn}$ may thus require an iterative approach wherein we could update the estimates of the b_i and also improve the estimates of the σ_{x_i} by incorporating information from both the preparations. In practice, our test depends on the choice of m. Generally, m is not chosen to be large (as then the test may become less efficient for a specific alternative hypothesis).

We consider the bias correction for $k_n = O(n^{4/5})$ by applying Jackknife method for given data. First method is Jackknife subsampling from the n original data (We call it NBC). From the Bahadur-type expression of the conditional quantile (Gangopadhyay and Sen, 1993), for a subsampling rate p $(0 < p < 1)$,

$$\begin{aligned} E\left[\hat{\xi}_{n,k_n} - \hat{\xi}_{pn,k_{pn}}\right] &= \beta(\xi)\left((k_n/n)^2 - (k_{pn}/pn)^2\right) + R \\ &= \beta(\xi) n^{-2/5} t^2 \left(1 - p^{-2/5}\right) \end{aligned}$$

where

$$\max_{k_n \in I_n(a,b)} |R| = O(n^{-3/5} \log n), \quad a.s.,$$

for $k_n = tn^{4/5}$. Let M_n be the average $(\hat{\xi}_{n,k_n} - \hat{\xi}_{pn,k_{pn}})/(t^2(1-p^{-2/5}))$ over the several values of p's for given t. Then, we may use

$$\hat{\xi}_{k_n,n} - M_n \tag{3.15}$$

as a bias reduced estimator of the conditional quantile. Second method is Jackknife subsampling method from the k_n nearest neighborhood sample (We call it KBC). For a subsampling rate p $(0 < p < 1)$, we have

$$E\left[\hat{\xi}_{n,k_n} - \hat{\xi}_{n,pk_n}\right] = \beta(\xi)(k_n/n)^2(1-p^2) + R,$$

where

$$\max_{k_n \in I_n(a,b)} |R| = O(n^{-3/5} \log n), \quad a.s.$$

We may use

$$\hat{\xi}_{n,k_n} - M_k \tag{3.16}$$

as a bias reduced estimator of the conditional quatile, where M_k is the average of $(\hat{\xi}_{n,k_n} - \hat{\xi}_{n,pk_n})/(1-p^2)$ over several values of p's.

4. Numerical Examples

In this section, we present some simulation results of the proposed conditional quantile estimators and the quadratic function, along with an application of our methods to a real data.

4.1 *Simulation results.* For the simulation study, we use the four-parameter logistic function model considered by Normolle (1991) whose parametric form is given by

$$Y(x) = \delta + \frac{\alpha - \delta}{1 + (x/\gamma)^{\beta}}.$$

We put $\alpha = 1000$, $\delta = 100$, $\gamma = 1$ and $\beta = 4$. We generate $x_i = exp(\phi_i/2)$ where ϕ_i is i.i.d. random numbers generated form $N(0, 1)$. And we let

$$Y^S(x) = Y^T(x - 0.5),$$

where ξ^S and ξ^T are the simulated response for the standard and test preparations, respectively. And we investigate the behavior of

$$k_n^{1/2} \left(\hat{\xi}_{nk}^T(x_i) - \hat{\xi}_{nk}^S(x_i + 0.5) \right), \tag{4.1}$$

and

$$Q = \sum_{i=1}^{m} k_n \left(\hat{\xi}_{nk}^T(x_i) - \hat{\xi}_{nk}^S(x_i + 0.5) \right)^2 / \hat{\sigma_i}^2, \tag{4.2}$$

where $\hat{\sigma_i}^2$ is the bootstrap estimator of $Var(\hat{\xi}_{nk}^T(x_i) - \hat{\xi}_{nk}^S(x_i + 0.5))$ for $k_n = [n^{4/5}]$.

We did the simulation study for $n = 50$ and 100. In the real bio-assays, sample size 50 or 100 is relatively large. But we need sample size at least 50 because of our nonparametric setups. Figure 1 is the Quantile to Quantile plot of (4.1) versus normal distribution, the box plot and the histograms. We check the Shapiro-Wilk test for normality test. P-values of (4.1) are 0.7914 and 0.2444 for $n = 50$ and 100, respectively. The Q values of (4.2) are 0.1031117 and 0.0099244 for $n = 50$ and $n = 100$ and the probabilities that χ_2^2 is less than Q is 0.050252 and 0.0049499 for $n = 50$ and 100, respectively, where χ_2^2 is the χ^2 distribution of 2 degree of freedom. All the simulation results show that the asymptotic properties work nicely for $n = 50$ and 100.

4.2 *Application to ARIC study.* In this section, we present a numerical study of our method applied to an actual study data. For the convenience, we use $p = 1/2$ for the examples: one can choose any p, $(0 < p < 1)$ in the context of particular study. Atherosclerosis Risk in Communities (ARIC) is a prospective investigation of the etiology and natural history of atherosclerosis and the etiology of clinical atherosclerotic disease in four US communities (Forsyth County, North Carolina; Jackson, Mississippi; suburbs of Minneapolis Minnesota; and Washington County, Maryland) (The ARIC Investigators (1989)). Examinations included ultrasound scanning of carotid artery. The data for the example included black female participants with second visits from all four communities. The response variable is

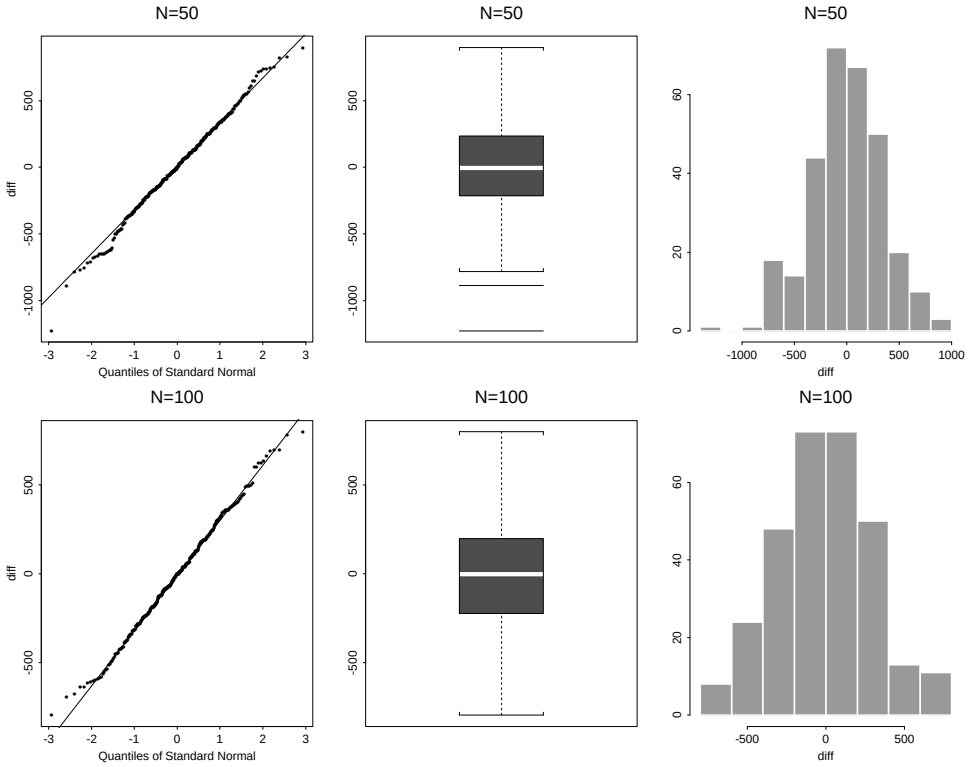

Figure 1: Q-Q plots, box plots and histograms of Q

the average carotid artery wall thickness(WT) scanned by Beta-mode ultrasound equipment. The explanatory variable is Body Mass Index (BMI) which is defined by weight/height^2 (Kg/m^2). The group variable is the Hypertension medicine history which is one if the participant has taken hypertension lowering medication in past two weeks, zero otherwise. The main interest is to test the equivalence of the carotid artery wall thickness between the two groups and to estimate the relative potency. The sample sizes are 623 and 735 for the hypertension and the normotension groups, respectively.

Table 1 shows the results of estimating the conditional median carotid artery wall thickness conditioned on the mean BMI of the blacks (30.162 Kg/m^2). All the variance estimators are calculated by the bootstrap method of 200 iterations. The δNN means $k_n = O(n^{4/5-2\delta})$ and the NN means $k_n = O(n^{4/5})$. The 95 % confidence interval of the difference between two medians of the δNN estimators includes zero; therefore, we cannot reject the null hypothesis that the conditional medians are the same between the hypertension and the normotension groups by 5% significance level. The differences between the δNN and the NN estimator for the hypertension and the normotension groups are 0.01159 and 0.01867. Jackknife bias

corrections (KBC (bias correction on the k_n samples) and NBC (bias correction on the whole n data)) were employed. Tables 2 and 3 show the estimated bias correction as a function of p (sub-sampling rate). There is no standard way to choose a particular p, so we tried several possible cases and used the mean for our correction. The 95% C.I.'s of the differences between the two medians do not include zero for both bias correction methods. Therefore, the results of the NN method with bias correction show that the median carotid artery wall thickness of hypertensive patients is significantly thicker than that of normotensive people after adjusting for BMI.

Table 1. ESTIMATED CONDITIONAL MEDIAN CAROTID ARTERY WALL THICKNESS CONDITIONED ON BMI=30.162

		Normotension	Hypertension
	n	623	735
	$k_n = n^{4/5-2\delta}$	90	101
δNN	$\hat{\xi}_1$	0.62965	0.66521
	$\hat{\sigma}$	0.01553	0.02406
	95% C.I.	(0.59921,0.66009)	(0.61805,0.71237)
	95% C.I. of the diff.	(-0.02057,0.09169)	
	$k_n = n^{4/5}$	172	196
NN	$\hat{\xi}_2$	0.64124	0.68388
	$\hat{\sigma}$	0.006596	0.006399
	$\hat{\xi}_2 - \hat{\xi}_1$	0.01159	0.01867
NBC	bias correction on k_n	0.00958	0.01207
	95% C.I.	(0.61878,0.64454)	(0.65927,0.68435)
	95% C.I. of the diff.	(0.02214,0.05816)	
KBC	bias correction on n	0.01870	0.01098
	95% C.I.	(0.60961,0.63547)	(0.66036,0.68544)
	95% C.I. of the diff.	(0.03235,0.06837)	

Table 2. JACKKNIFE BIAS CORRECTION ON k_n SAMPLE

	Normotension	Hypertension
p	$k_n = n^{4/5}$=172	$k_n = n^{4/5}$= 196
0.90	0.00000	0.00000
0.80	0.01981	-0.00133
0.70	0.01527	-0.02294
0.60	0.01811	-0.00075
0.50	0.01823	0.01404
0.40	0.01589	0.02483
0.30	0.02055	0.02292
0.20	0.01424	0.02173
0.10	0.02751	0.02896
mean	0.01870	0.01098

To test the bioequivalence between the two groups and to estimate the relative potency, we assume the fundamental assumption of an assay :

$$\xi^T(x) - \xi^S(x+\mu) = 0 \text{ for all } x \in \mathcal{X}, \tag{4.3}$$

where $\xi^S(\cdot)$ and $\xi^T(\cdot)$ are the conditional medians of carotid wall thickness given BMI ($= x$) for normotension and hypertension groups, respectively, and $\mathcal{X}$ is a closed interval on the real line. We need to check the parallelism before doing any

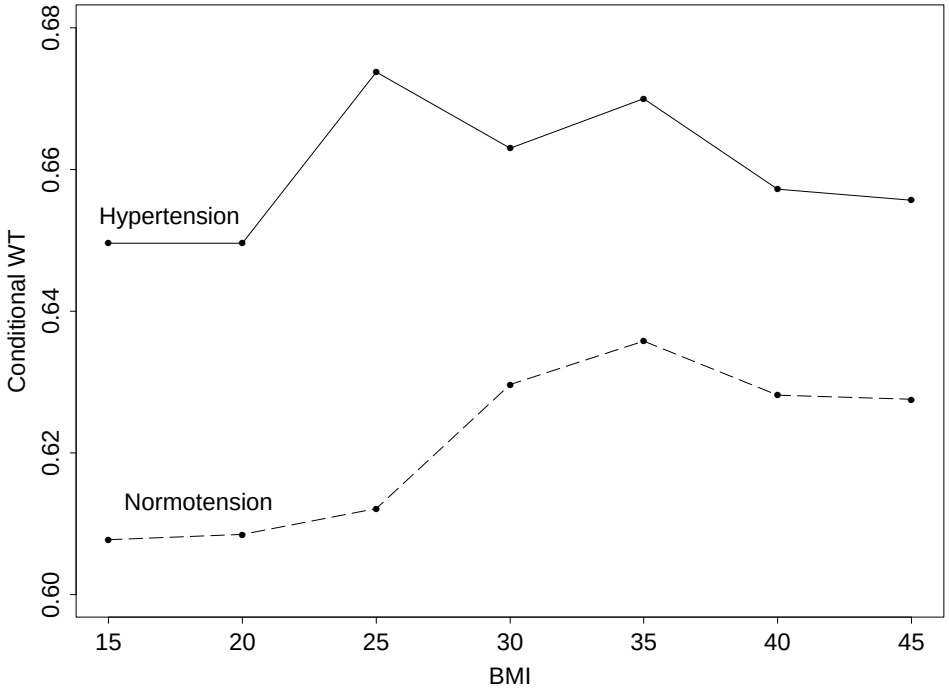

Figure 2: Estimated conditional median carotid artery wall thickness conditioned on BMI for two groups

Table 3. JACKKNIFE BIAS CORRECTION ON THE WHOLE n DATA

		Normotension n =623			Hypertensi on n =735		
# of gps	p	pn	k_{pn}	bias	pn	k_{pn}	bias
10	0.1000	62.3	27	0.02517	72.3	31	0.01740
9	0.1111	69.2	30	0.00402	80.3	33	0.01586
8	0.1250	77.9	33	0.01081	90.3	37	0.00702
7	0.1429	89.0	36	0.00757	103.3	41	0.02390
6	0.1667	103.8	41	0.00449	120.5	46	0.00586
5	0.2000	124.6	48	0.01069	144.6	53	0.00793
4	0.2500	155.8	57	0.00432	180.8	64	0.00655
mean				0.00958			0.01207

statistical inferences based on the assumption (4.3). Figure 2 shows the estimated conditional median curves over BMI, and we decided that there is no violation of the parallelism. For the statistical inferences, we use $\boldsymbol{x}' = (15, 20, 25, 30, 35, 40, 45)$ and 200 bootstrap iterations to estimate the variance. To estimate μ, we applied

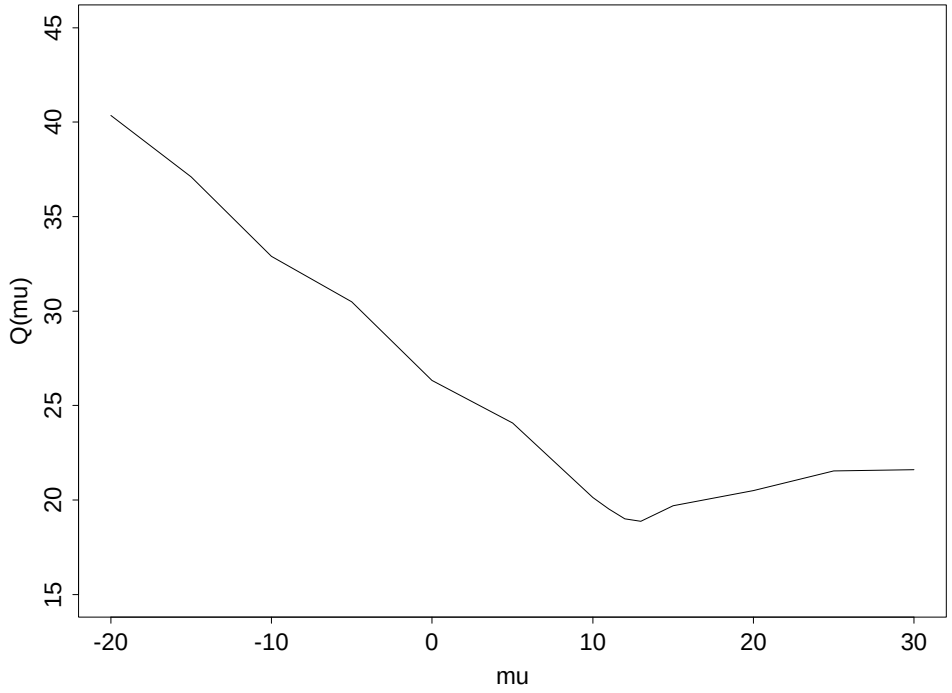

Figure 3: $Q_m(\mu)$ vs. μ

the minimum chi-square rule as discussed in section 3. Figure 3 shows

$$Q_m(\mu) = \sum_{i=1}^{m} \left(\hat{\xi}^T_{n,k_n}(x_i) - \hat{\xi}^S_{n,k_n}(x_i + \mu) \right)^2 / 2\tilde{\sigma}_{ii} \tag{4.4}$$

where $\tilde{\sigma}_{ii}$ is the bootstrap estimator of the variance of $\hat{\xi}^T_{n,k_n}(x_i)$ as a function of μ. From the figure, we estimate μ to be 13 which minimizes (4.4), i.e., the WT curve of hypertension patients is shifted to the left by 13 units of BMI compared to the WT curve of the normotension group. For testing (4.3), we have $Q(\hat{\mu}) = 18.87$ with $p = 0.005$. Therefore, we reject the hypothesis (4.3) which means the difference between the two WT curves are statistically significant.

The difference of the median WT's between the two groups is 0.04974. We subtract the difference from the hypertension group and apply the test again. The values $Q = 5.88$ with $p = 0.55$ were obtained. Our conclusions are as follows: 1) the WT curve of the hypertension group is significantly higher than that of the normotension group for the entire range of BMI, 2) but hypertension effect-adjusted WT curves are equivalent for the two groups; WT curves have the same shape for the two groups.

In passing, we may note that to test the fundamental assumption (3.8), we could use the Kolmogorov-Smirnov norm:

$$\sup_{x\in\mathcal{X}} |W^T(x) - W^S(x+\mu)|$$

and choose an estimator that minimizes the above (with respect to μ). In that way, we could avoid the arbitrariness in the choice of the m points $x_1, \ldots, x_m$ that we have prescribed in the proposed minimum chi-squared type estimator. The estimator of the relative potency may possess some appealing properties such as asymptotic normality or consistency. But that generally requires larger sample sizes and more sophisticated statistical analysis. One other interesting question is how to select k-NN samples for the processes. In this paper, we select x's by the same increment over the range of interest. We may select exclusive samples to guarantee independence among them, or we may allow some dependency by selecting not totally exclusive samples.

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