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Nonparametric Stepwise Procedure for Identification of Maximum Safe Dose (MSD)

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Authors' contributions

This work was carried out in collaboration between all authors. Author MJA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors NKH and AL managed the analyses of the study. Author AL managed the literature searches. All authors read and approved the final manuscript.

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Abstract

In this article we consider identification of maximum safe dose (MSD) in a dose response study for distribution-free endpoints. Where the maximum safe dose is the highest dose level that does not exceed the median toxicity of the zero dose by a predetermined margin. A nonparametric confidence set-based approach was proposed, that is we incorporate Mann-Whitney method with the partitioning principle in a step-down fashion for safety evaluation. A comprehensive study of the familywise error rate for our new procedure was compared with the dose response (DR) procedure via a Monte Carlo simulations. An example from preclinical trial in genetic toxicology was used for illustrative purpose.

Keywords: Maximum safe dose; partitioning principle; distribution-free endpoint; familywise error rate; confidence set.

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1 Introduction

A toxic assessment of a new drug or a pharmaceutical compound is one of the most vital concerns to toxicologist and regulatory agencies e.g. Food and Drug Administration (FDA).The safety of our food, environment and pharmaceutical drugs is a critical issues in toxicological investigation.

The major challenge in statistical procedures design for dose response study for safety assessment is the control of consumer risk, that is, the probability of erroneously concluding on safety. Investigations that rely on estimating confidence intervals of parameters involved, guarantee significant control of familywise error rate than its corresponding P-values. As a result, the international conference on harmonization (ICH) E9 guidelines0 [1] in clinical trials requires that estimates of treatment effect should be accompanied by confidence intervals, whenever possible, and the way in which these should be calculated should be identified. Furthermore, the International committee of medical journal Editors (ICMJE) [2] made the following statement in that same direction: *when possible, quantitative finding and present with appropriate indicators of measurement errors or uncertainty (such as confidence intervals). Avoid relying solely on statistical hypothesis test in such as P- values which fails to convey important quantitative information* .A serious difficulty with this requirement is that there is no established procedure for construction confidence intervals in multiple comparisons procedures especially in nonparametric settings.

The identification of maximum safe Dose (MSD) has been studied by many authors for safety endpoint e.g. [3-6] but they do not estimate simultaneous confidence intervals as recommended by [1]. Simultaneous confidence intervals approaches were investigated by [7] for a normally distributed endpoints for ratios, [8] for Gaussian distributed endpoints. [9] proposed stepwise confidence interval for identification of minimum effective dose (MED) and maximum safe dose without multiplicity adjustment based on partitioning principle [10] for normally distributed endpoints. After this proposal, they have been several extensions for construction of stepwise confidence intervals without multiplicity adjustment for different settings: [11] for ratios, [12] for binary data endpoint, [13] for different between two poisson rate.

None of these investigators employ purely nonparametric methods. On the other hand, in practice, there is a need for compatible simultaneous confidence intervals when the normal assumptions are violated, sometime for a skewed data, continuous and discontinuous distributions [14]. This work is therefore, focusing on construction simultaneous nonparametric upper bound procedure based on Wilcoxon Mann-Whitney test incorporating the partitioning principle for confidence sets procedure proposed by [9] for estimating MSD. Hence the paper is concern with the safety assessment of chemical compounds when the toxicological endpoint is not normally distributed within a predetermined safety margin.

This article is organized as follows. Section 2, is the preliminary: it gives an overview of Mann-Whitney Test. In section 3, the proposed procedure is formulated and a testing procedure for construction of confidence intervals is derived. Section 4 delineate construction of stepwise confidence intervals based on Mann - Whitney statistics for inferences of MSD. In section 5, a proposition is stated and proved for the main result of this article. Section 6 illustrate an example on genetic toxicology and Monte Carlo simulations was performed to confirm theoretical result and finally in section 7, concludes the article with a definitive proposition with the assertion that the proposed stepwise confidence intervals procedure strongly control the familywise error rate in a strong sense for MSD identification in nonparametric settings.

2 Preliminary Review Study

2.1 Mann-Whitney test

Considering a review of Mann-whitney statistical test [15], we can construct stepwise confidence sets procedure for nonparametric data sets. Suppose that F and G are two independent continuous cumulative distribution functions but they have similar in shape, we randomly sampled $x_1, x_2, \cdots, x_n \in G$ and

$y_1, y_2, \cdots, y_m \in F$ respectively. Consider a location shift parameter $\Delta = \text{Med}(Y) - \text{Med}(X)$ the median difference between the two samples such that $G(x) = F(x - \Delta)$ for all values x and 0. Our purpose is to evaluate the hypothesis that two samples come from the same population against the alternative that G is stochastically larger than F. that is:

$$H_0 : \Delta = 0 \text{ versus } H_1 : \Delta > 0$$

Mann-Whitney defined the U statistics as:

$$U = \sum_{i=0}^{m} \sum_{j=0}^{n} \varphi(X_i, Y_j)$$

Where

$$\varphi(X_i, Y_j) = \begin{cases} 1, & if\ Y_j > X_i \\ 0, & otherwise \end{cases}$$

The confidence limits for $U^1 \leq U^2 \leq \cdots \leq U^{mn}$ from the smallest to the largest. The upper confidence bound for Δ, the location shift parameter according to [16] is:

$$\Delta_U = (-\infty, U^{mn+1-Ca}) = U^{mn+1-Ca}$$

Where $C_a = \frac{n(2m+n+1)}{2} + 1 - \omega_a$ is the C_ath in position in the list of mn increasing in the differences $Y_j - X_i (U^1 \leq U^2 \leq \cdots \leq U^{mn})$and ω_a is the upper α percentile of the null distribution of the Wilcoxon Rank Sum Statistic. Hence we propose a new procedure by using partitioning principle and incorporating into Mann-Whitney statistic for construction of stepwise confidence sets for nonparametric endpoint settings.

2.2 The proposed procedure

Let $i = 1, \cdots, k$ representing increasing in dose levels for the new treatments groups, where $i = 0$ is the index of the control dose group. We consider one-way layout:

$$X_{ij} = \xi_i + \epsilon_{ij} \text{ with } i = 0, 1, \cdots, k \text{ and } j = 1, \cdots, n_i$$

Where X_{ij} be the observed response to toxicity of the jth subjects in the ith group. The random errors ϵ_{ij}, $i = 0, 1, \cdots, k$ and $j = 1, \cdots, n_i$ are independent and identically distributed continuous variables, whilst ξ_i are unknown median effect. In this set up, our main goal is to identify the maximum dose level producing a desirable toxicity over that of zero -dose control which is referred to as maximum safe dose (MSD). We assumed that a higher dose cannot be scan as toxic if a lower dose is not scan as toxic (Note that toxicity increases with increasing in dose levels). This implies any dose less or equal to MSD is safe and any dose level higher than MSD is unsafe.

2.3 Testing procedure

In designing an experiment to assess the toxicity of a drug candidate, we make inferences about location parameter {median) vector of interest $\theta = (\xi_0, \xi_1, \xi_2, \cdots, \xi_k)$, and then construct a stepwise confidence set procedure in one-way layout for $\theta_{i0} = \xi_i - \xi_0$ for all $i = 1,2, \cdots, k$ such that coverage probability is at least $1 - \alpha$. We avert the inference that $\theta_{i0} < \delta$ if and only if the probability making at least one incorrect rejection in the family of hypotheses $H_i^{(2)}: \xi_1 - \xi_0 \geq \delta$ for all $i = 1, 2, \cdots, k$ is not greater than α is achieved.

This achievement will guarantee significant protection against incorrect decision. Hence strongly controlling the family-wise error rate is a critical requirement in multiple comparison procedures in dose - finding.

MSD is defined as the highest experimental dose with no significantly increased safety effect relative to placebo or control group if the dose - response relationship is continuous. That is:

$$\text{MSD} = \max\{\text{i}: \xi_{\text{i}} < \xi_0 + \delta\}$$

Food and Drug Administration (FDA)'s criterion for safety consists of the proof statistical significance and proof of clinical relevant:

$\text{H}_{\text{i}}^{(2)}: \xi_1 - \xi_0 \geq \delta$ (substance is unsafe under test conditions)

$\text{A}_{\text{i}}^{(2)}: \xi_1 - \xi_0 < \delta$ (substance is safe under test conditions)

for any $\text{i} = 1, 2, \cdots, \text{k}$

Where δ denote the threshold value and the interval $(-\infty, \delta), \delta > 0$ the pre-specified safety range.

3 A Stepwise Confidence Intervals Procedure Based on Mann- Whitney Statistics for Inferences of MSD

Firstly, compute the upper limits:

$$\Delta_U = \left(-\infty, U_i^{\gamma}\right) for\ i = 1, 2, \cdots, k$$

where $\gamma = n_i n_0 + 1 - C_a \ and\ C_a = \frac{n_0(2n_i + n_0 + 1)}{2} + 1 - \mathcal{W}_a$, the $\mathcal{W}_a$ is the Wilcoxon Rank Sum Statistics and k is the total number of doses to be tested . Secondly, we start the scan by first scanning the lowest dose level (that is for $i = 1$,) , and sequentially scan the other doses $i = 2, 3, \cdots, k-1, k$ without adjusting the α levels in ascending manner searching for the first integer M(where a statistically insignificant treatment effect occur) if it exist such that $U_M^{\gamma} > \delta \ and\ U_{M+1}^{\gamma} > \delta$.

Hence, in this set up, the dose level at M is identified as MSD. If $U_1^{\gamma} > \delta$ then no MSD can be identified and none of the doses can be scanned as safe. If $U_k^{\gamma} < \delta$. It is an indication that no toxicity exist and all doses are safe. Once dose M is estimated as $\widehat{MSD}$, then the upper confidence intervals for doses $M+2, M+3, M+4, \cdots, k$ are unnecessary and should not be computed. In other words, If $H_i^{(2)}$ is significant and $H_{(i+1)}^2$ is insignificant then there is strong evidence that only doses $1, 2, \cdots, i$ are safe. Hence patients are not subjected on doses $i+1, i+2, \cdots, k$. Consequently, the distinguish feature about this method is that, it reduces the risk of unnecessary early exposure of patients undergoing clinical trials to possible toxic effect of the new drug or the compound. Notice that multiplicity adjustment is not needed in this stepwise procedure.

4 Main Results

Proposition 4.1.

Consider a parameter of comparisons of the median differences $\theta_{i0} = \xi_i - \xi_0, 1 \leq i \leq k$ for an independent nonparametric data x_{ij} , where $i = 1, 2, \cdots, k$ and $j = 1, 2, \cdots, n_i$ and ξ_0, ξ_1 be the median of the ith treatments and the control median respectively. Let $\theta = \{\xi_0, \xi_1, \cdots, \xi_k\}$ be vector of medians effect of the

$k+1$ *different observations. For any* $i = 1, 2, \cdots, k$ *let* U_i^{γ} *be the* $100(1-\alpha)\%$ *confidence upper limit for* $\xi_i - \xi_0$ *, where* U_i^{γ} *is the value at the* γth *position in the list of* $n_i n_0$ *increasing in ordered differences between the treatment groups and control group and* $\gamma = n_i n_0 + 1 - C_a$ *and* $C_a = \frac{n_0(2n_i+n_o+1)}{2} + 1 - \omega_a$ *the is* ω_a *the upper* α *percentile of the null distribution of Wilcoxon Rank Sum Statistics. Denote M, the first integer* i *such that* $U_i^{\gamma} \prec \delta$ *if such an* $i(1 \leq i \leq k)$ *exist. Otherwise Let* $M = k+1$*. Then for the parameter space* $\theta \in \Theta$*.*

$$P(\bigcap_{i=1}^{M-1}(\theta_{i0} < \delta) \cap \delta \leq U_{(M)}^{\gamma}) \geq 1 - a$$

The proof is based on application of theorem 1 in [9]:

Note that simultaneous confidence sets for $\xi_i - \xi_0$ of the form $C_i(X) = (-\infty, U_i^{\gamma})$ for $i = 1, 2, \cdots, k$ are directed toward $\xi_i - \xi_0 < \delta$. Also $C_M(X) = (-\infty, U_i^{\gamma})$ and $U_{K+1}^{\gamma} = \infty$.

This guarantees a significant protection against incorrect decision because, the overall coverage probability is not less than $1 - a$. Controlling the familywise error rate (FWER) in a strong sense when testing simultaneously a family of hypothesis is a critical issue in multiple comparisons procedures, especially in dose findings. [17], pointed out that, the probability of at least one type 1 error rate should be kept at or less than pre-specified level $1 - a$. That is the probability of erroneously declaring a toxic dose as safe at a pre-specified level. Which is

$$P(\ declaring\ unsafe\ dose\ as\ safe) \leq \alpha$$

That is:

$$P\{\widehat{MSD} < MSD\} \leq \alpha$$

Consequently, we state and proof the following proposition:

Proposition 4.2. *The simultaneous nonparametric stepwise procedure strongly controls the familywise error rate (FWER) at level α*

proof Let I be any unknown subset of $\{1, 2, \cdots, k\}$. Suppose that $I = \emptyset$ then no familywise error rate exist. Thus assume that $I \neq \emptyset\ and\ I = \{i_1, i_2, \cdots, i_m\}$, where

$1 \leq i_1 < i_2 < \cdots < i_m \leq k$, then

$$P\big(Reject\ one\ of\ H_i^{(2)}, i \in I \big| H_i^2, i \in I\ is\ true\big)$$

$$\begin{aligned}
&= 1 - P\big(Do\ not\ reject\ all\ H_i^{(2)}, i \in I \big| H_i^{(2)}, i \in I\ is\ true\big) \\
&\leq 1 - P\big(Do\ not\ reject\ H_{i_m}^{(2)}, i \in I \big| H_i^{(2)}, i \in I\big) \\
&= 1 - P\ (\{U_{i_m} \not\subset (-\infty, U_{i_m}^{\gamma})\} |\ H_i^{(2)}, i \in I) \\
&\leq 1 - P(\theta_{i_1 0} < \delta, \theta_{i_2 0} < \delta, \cdots, \theta_{i_{(M-1)}0} < \delta, \theta_{i_{(M)}0} \leq U_{i_m M}^{\gamma}) \\
&\leq 1 - (1 - a)\ \text{(By Proposition 4.1)} \\
&= \alpha
\end{aligned}$$

4.1 An example: On genetic toxicology

In today's world, the human race is increasingly exposed to potential adverse effect as a result of significant increase in the number of novel chemicals, their spread in the environment, and ingestion of these through food. These include food additives, agrochemicals, industrial chemicals, solvents, drugs, etc. Most of these chemical substances cannot be said to be free of mutagenic/carcinogenic agents with certainty. In fact, the Introduction of the *salmonella* assay by [18] has led to a startling revelations that human activities and our environment are replete with mutagenic activity: these include cigarette smoke [19], urban air [20,21], river water [22], drinking water [23], food [24] , soil [25], and house dust [26]. This revelation has triggered much public concern among regulatory agencies, health authorities and the academia about the potential risk the current and future generations are posed to. Mutagens are chemical or compounds causing chemical or physical genetic alteration (e.g. DNA structure) in man. These have serious potential health risk to society, hence understanding mutagenic/carcinogenic effect of chemicals is very important. To protect the human race against exposure to mutagenic compound is a safety study in genetic toxicology.

Genetic toxicology test are used to predict mutagenic or carcinogenic potential of chemical compounds for regulatory purposes. The fundamental concerns for regulatory agencies in genetic toxicology is the risk to future generations. The short-term test (SST) is the most widely used Ames assay in genotoxicity evaluation, this is because the long-term bioassay is time consuming, costly and require adequate physical facilities. The SST is based on detecting mutated histidine-depended cell strain of *salmonella typhinurium* (e.g. TA1535, TA1537, TA97/TA97a, TA98 etc). The mutated bacteria can mutate back to the wild type if exposed to mutagenic compound.

Statistical methodologies in evaluation of Ames test data are fraught with many challenges, a comprehensive discussion of these problems are detailed in [27,28], generally, the major problems pointed out by these authors are overdispersion and the difficulty in assigning specific discrete distribution to the count data.

The endpoint in mutagenicity data is number of revertants colonies counted at each replicated plates. The primary concern in evaluation of Ames test data is the control of the probability of erroneously concluding on safety (consumer risk), that is the control of the familywise error rate (FWER)

For illustrative purposes for our new procedure, we obtain a data from [28].

We determine maximum safe dose level by considering Table 1 , the 95% one -sided upper confidence bound for median differences $\xi_i - \xi_0$ for $i = 5, 15, 50, 150$ and 500 dose levels, the cut off values are respectively $U_5^Y = 2, U_{15}^Y = 2, U_{50}^Y = -2, U_{150}^Y = -2, U_{500}^Y = 4$. For illustrative purposes, we set the clinical relevant threshold value.

Table 1. Revertant count of TA1535 strain without metabolic activation

Doses (in μg)	Number or Revertants
0	16 17 17 20 18
5	18 18 19
15	16 20 20
50	20 24 28
150	26 28 20
500	16 20 16

Table 2. Inference on maximum safe dose from Table 1

Median difference	95% upper bound
$\xi_5 - \xi_0$	$(-\infty, 2)$
$\xi_{15} - \xi_0$	$(-\infty, 2)$
$\xi_{50} - \xi_0$	$(-\infty, -2)$
$\xi_{150} - \xi_0$	$(-\infty, -2)$
$\xi_{500} - \xi_0$	$(-\infty, 4)$

$\delta = 1$ .In other words, the safety of a chemical compound is acceptable up to $\delta = 1$ level. This implies all $U_i^{\gamma} < 1$ are clinically or biologically significant. Because toxicity or adverse effect of a pharmaceutical compound increasing with dose levels. We start from the lowest dose level. This is because the lowest dose level is virtually free of adverse effect..

The new procedure can be applied in a stepwise fashion as follows:

Step 1, We compare the lowest dose levels (5) with that of the zero dose control (0). That is $\xi_5 - \xi_0$. Notice from Table 2 that $U_5^{\gamma} = 2 \not< 1$ This means that when we consider $(-\infty, U_5^{\gamma}) = (-\infty, 2) \not\subset (-\infty, 1)$, we can inferred that $\xi_5 - \xi_0 \subset (-\infty, 2)$ and conclude that MSD cannot be estimated,consequently the procedure is terminated at **step 1**. This implies, all doses can be declared as unsafe. However, according to [11] since the new procedure rely on pairwise comparisons, the initial assumption of prioritization can be violated the only requirement is that the dose levels should be contiguous. We start from $U_{50}^{\gamma} = -2 < 1$, $U_{150}^{\gamma} = -2 < 1$ and $U_{15}^{\gamma} = 2 > 1$ then the procedure stop and we declare the MSD as 150. In other words, dose levels at 50 and 150 are safed, that is, they have no mutagenic effect and the rest of doses are mutagenic. The result is consistent with [11].

4.2 Simulation study for identification of MSD

To implement the new procedure, we investigate the familywise error rate (FWER). This investigation was performed for comparing k=3 and k=5 treatment with control. To ensure high precision, the number of iteration was set to 10,000. A multivariate normal distribution was generated with zero correlation and unit variance, we use R- software package exact RankTest to do our simulations. For illustrative purposes we set our threshold δ = 1.5, 2. and 2.5. Actually, in practice these threshold are predetermined by clinical experts.

In estimating the familywise error rate in identifying the maximum safe dose we compared the dose-response (DR) procedure proposed by [9] and our new procedure (NP). We consider only situation of balanced sample sizes set at $n = 27, 30\ 35, 40\ and\ 45$. The $\widehat{MSD} = \max\{\xi_i < \xi_0 + \delta\ for\ i = 1, 2, \cdots, k\}$. Three different types of dose-response relationship namely, linear response function, Step response function and umbrella response function were examined. From Tables 3 and 4 with their respective $k = 3\ and\ k = 5$ treatments, and the $a = 0.05$ for sample size 27, the estimated FWER for most of the configurations exceeded $0.0543 = 0.05 + \sqrt{0.05 \times 0.95 / 10{,}000}$ for the NP procedure, which is a clear indication that the new procedure is invalid and do not control the familywise error rate, but that of the DR procedure control the FWER in the strong sense when n= 27.

We consider Tables 5 and 6 for both $k = 3\ and\ k = 5$ treatments respectively with the sample size n = 30 and α = 0.05.

Firstly, let consider an example where k=3, we consider a linearly increasing median response function $\theta = (\xi_0, \xi_1, \xi_2, \xi_3) = \{8, 9, 10, 11\}\ for\ \delta = 1.5$. We infer that $\widehat{MSD} = \max\{\xi_i < \xi_0 + \delta\} = 1$ and the familywise error rate (FWER) are 0.0144 for the DR procedure and 0.0181 for NP procedure respectively. We examined step median response function $\theta = (\xi_0, \xi_1, \xi_2, \xi_3) = \{12, 13, 13, 13\}\ for\ \delta = 2.5$. We infer

that $\widehat{MSD} = \max\left\{\xi_i < \xi_0 + \delta\right\} = 2$ and familywise error rate (FWER) is 0.0262 for the DR procedure and 0.0458 for NP procedure respectively. Consider an umbrella response function $\theta = (\xi_0, \xi_1, \xi_2, \xi_3) = \{11, 12, 14, 13\}\ for\ \delta = 1.5$. We infer that estimated $\widehat{MSD} = \max\left\{\xi_i < \xi_0 + \delta\right\} = 1$ and the familywise error rate (FWER) are 0.0140 for DR procedure and the 0.0194 for NP procedure respectively. And also $\theta = (\xi_0, \xi_1, \xi_2, \xi_3) = \{10, 11, 14, 12\}\ for\ \delta = 2$. We infer that $\widehat{MSD} = \max\left\{\xi_i < \xi_0 + \delta\right\} = 1$ and the familywise error rate (FWER) are 0.0153 for DR procedure and 0.0185 for NP procedure respectively.

We consider Table 7 in this case $k = 5$ treatments. We investigate linearly increasing median response $\theta = (\xi_0, \xi_1, \xi_2, \xi_3, \xi_4, \xi_5) = \{10, 11, 12, 13, 14, 17\}\ for\ \delta = 2.5$ $\widehat{MSD} = \max\left\{\xi_i < \xi_0 + \delta\right\} = 1$ the FWER are 0.0131 for DR procedure and 0.0192 for NP procedure $for\ \delta$ the FWER are e respectively .We also study step median response function in Table 8 for $\theta = (\xi_0, \xi_1, \xi_2, \xi_3, \xi_4, \xi_5) = \{10, 11, 11, 11, 12, 12\ for\ \delta=1$ we conclude that the estimated $MSD=\max \xi_i < \xi 0+\delta=1$ and the FWER are 0.0167 for DR procedure and 0.0217 for NP procedure respectively. In addition to the step median response function, we examined another step median response function $\theta = (\xi_0, \xi_1, \xi_2, \xi_3, \xi_4, \xi_5) = \{10, 11, 11, 11, 13, 13\ for\ \delta=1.5$. We claim that the estimated $MSD=\max \xi_i < \xi 0+\delta=2$ and the FWER are 0.0167 for the DR procedure and 0.0235 for the NP procedure respectively. For the umbrella median response function $\theta = (\xi_0, \xi_1, \xi_2, \xi_3, \xi_4, \xi_5) = \{11, 12, 13, 17, 15, 12\}\ for\ \delta = 2$ We infer that $\widehat{MSD} = \max\left\{\xi_i < \xi_0 + \delta=2\right.$ and the FWER are 0.0141 for DR procedure and 0.0320 for the NP procedure respectively.

Table 3. Estimated FWER for $\alpha = 0.05, k = 3, n_1 = n_2, = n_3, = n_4$=27

Configuration	$\xi_0 \xi_1, \xi_2, \xi_3$	δ	MSD	DR	NP
Linear Function	8, 9, 10, 11	1.5	1	0.0221	0.0312
	11, 12, 13, 14	2	1	0.0232	0.0282
	13, 14, 15, 16	2.5	1	0.0232	0.0318
Step Function	13, 14, 14, 14	1.5	3	0.0459	0.0749
	12, 14, 14, 15	2	3	0.0407	0.0590
	12, 13, 13, 13	2.5	2	0.0421	0.0842
Umbrella Function	11, 12, 14, 13	1.5	3	0.0227	0.0311
	10, 11, 14, 12	2	1	0.0270	0.0295
	11, 12, 16, 13	2.5	1	0.0230	0.0295

Table 4. Estimated FWER for $\alpha = 0.05, k = 5, n_1 = n_2, = n_3, = n_4 = n_5$=27

Configuration	ξ_0 , ξ_1 , ξ_2 , ξ_3, ξ_4, ξ_5	δ	MSD	DR	NP
Linear Function	11, 12, 13, 14, 15, 17	1.5	1	0.0214	0.0288
	11, 12, 13, 14, 15, 16	2	1	0.0194	0.0316
	10, 11, 12, 13, 14, 17	2.5	1	0.0214	0.0337
Step Function	10, 11, 11, 11, 13, 13	1.5	1	0.0611	0.0699
	11, 12, 12, 12, 12, 13	2	1	0.0721	0.0963
	11, 12, 12, 12, 12, 15	2.5	1	0.0667	0.0878
Umbrella Function	11, 12, 17, 15, 13, 12	1.5	1	0.0237	0.0543
	11, 12, 13, 18, 13, 12	2	1	0.0220	0.0548
	11. 12, 18, 17, 15, 12	2.5	1	0.0209	0.0616

Table 5. Estimated FWER for $\alpha = 0.05, k = 3, n_1 = n_2, = n_3, = n_4$=30

Configuration	$\xi_0, \xi_1, \xi_2, \xi_3$	δ	MSD	DR	NP
Linear Function	8, 9, 10, 11	1.5	1	0.0144	0.0181
	11, 12, 13, 14	2	1	0.036	0.0192
	13, 14, 15, 16	2.5	2	0.0127	0.0163
Step Function	13, 14, 14, 14	1.5	1	0.0255	0.0471
	12, 14, 14, 15	2	3	0.0266	0.0350
	12, 13, 13, 13	2.5	2	0.0262	0.0458
Umbrella Function	11, 12, 14, 13	1.5	1	0.0140	0.0194
	10, 11, 14, 12	2	1	0.0153	0.0185
	11, 12, 16, 13	2.5	3	0.0123	0.0181

Table 6. Estimated FWER for $\alpha = 0.05, k = 5, n_1 = n_2, = n_3, = n_4 = n_5$=30

Configuration	$\xi_0 \xi_1, \xi_2, \xi_3, \xi_4, \xi_5$	δ	MSD	DR	NP
Linear Function	11, 12, 13, 14, 15, 17	1.5	1	0.0145	0.0186
	11, 12, 13, 14, 15, 16	2	1	0.0122	0.0176
	10, 11, 12, 13, 14, 17	2.5	1	0.0131	0.0192
Step Function	10, 11, 11, 11, 13, 13	1.5	1	0.0167	0.0235
	11, 12, 12, 12, 12, 13	2	1	0.0185	0.0258
	11, 12, 12, 12, 12, 15	2.5	1	0.0206	0.0306
Umbrella Function	11, 12, 17, 15, 13, 12	1.5	1	0.0151	0.0343
	11, 12, 13, 18, 13, 12	2	1	0.0141	0.0320
	11. 12, 18, 17, 15, 12	2.5	1	0.0142	0.0370

Table 7. Estimated FWER for $\alpha = 0.05, k = 3, n_1 = n_2, = n_3, = n_4$=35

Configuration	$\xi_0 \xi_1, \xi_2, \xi_3$	δ	MSD	DR	NP
Linear Function	8, 9, 10, 11	1.5	1	0.0050	0.0099
	11, 12, 13, 14	2	1	0.0066	0.0073
	13, 14, 15, 16	2.5	2	0.0053	0.0090
Step Function	13, 14, 14, 14	1.5	3	0.0109	0.0226
	12, 14, 14, 15	2	3	0.0112	0.0145
	12, 13, 13, 13	2.5	2	0.0102	0.0245
Umbrella Function	11, 12, 14, 13	1.5	1	0.0398	0.0221
	10, 11, 14, 12	2	1	0.0415	0.0140
	11, 12, 16, 13	2.5	3	0.0429	0.0245

Table 8. Estimated FWER for $\alpha = 0.05, k = 5, n_1 = n_2, = n_3, = n_4 = n_5$=35

Configuration	$\xi_0, \xi_1, \xi_2, \xi_3, \xi_4, \xi_5$	δ	MSD	DR	NP
Linear Function	11, 12, 13, 14, 15, 17	1.5	1	0.0059	0.0070
	11, 12, 13, 14, 15, 16	2	1	0.0067	0.0095
	10, 11, 12, 13, 14, 17	2.5	2	0.0067	0.0081
Step Function	10, 11, 11, 11, 13, 13	1.5	1	0.0157	0.0235
	11, 12, 12, 12, 12, 13	2	1	0.0185	0.0258
	11, 12, 12, 12, 12, 15	2.5	1	0.0206	0.0306
Umbrella Function	11, 12, 17, 15, 13, 12	1.5	2	0.0072	0.0137
	11, 12, 13, 18, 13, 12	2	5	0.0063	0.0144
	11. 12, 18, 17, 15, 12	2.5	5	0.0062	0.0171

We conduct several simulations to compared the Type 1 familywise error rate, of our new procedure (NP) with that of DR procedure proposed by [9]. For illustration purposes, a real published data set example for

evaluation of genetic toxicology was used for identification of MSD by employing the new procedure(NP) and the DR procedure in pre-clinical settings.

We varied our sample sizes from $27 \le n \le 50$ in our study. In our simulation studies, it was revealed that identification of MSD under multivariate normal distribution, indicated that for all configuration DR perform better in controlling FWER than the NP for sample sizes $n < 30$. In fact, NP failed to control FWER for step and inverted umbrella configurations for the MSD investigations.

The performance of DR and NP were similar to for linearly response configurations in controlling FWER for sample sizes $30 \le n \le 40$. The simulations results suggested that, although NP perform better than DR after $n > 40$, it is highly conservative in controlling the FWER.

5 Conclusion

In randomized clinical trials, confidence interval procedures are prefer to P-vales, not only does it gives significant protection against incorrect decision but also provide relevant clinical quantitative information. According to International conference of Harmonization (ICH E9) and International committee of medical journal Editors (ICMJE) recommendations, inferences drawn from statistical procedures on dose-finding based on confidence intervals are clinically more relevant than P-values. Therefore this paper propose stepwise confidence set-based procedure for identification of MSD in randomized clinical trials for distribution-free endpoints. The proposed method discuss in this article could be extended to nonparametric stepwise procedure for identifying minimum effective dose and maximum safe dose simultaneously. In summary, the NP method is feasible for a situation of unknown distribution endpoints especially when the sample size $n > 30$. However, the DR procedure is recommended when the normal assumption is not violated.

Competing Interests

Authors have declared that no competing interests exist.

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