Clinical Communication

# Application of isotonic regression in estimating $ED_g$ and its 95% confidence interval by bootstrap method for a biased coin up-and-down sequential dose-finding design

## INTRODUCTION

Newer anaesthetic drugs introduced in practice and older drugs for novel route or indication has led to the need to determine the optimal dose. In this context, two most recent studies, for example, estimated the effective dose of opioids for epidural initiation in the latent and active phases during the first stage of labour and remimazolam bolus for anaesthesia induction in different age groups.[1,2] The primary aim of these studies was to estimate a quantile, a dose at which a desired probability of response is achieved. This dose is called an effective dose (ED) with quantile g; that is, $ED_g$ is defined as the dose of a drug that produces a response of interest at quantile 'g' ('g' may be 90, 95 or 99%) of the study population. In these studies, doses cannot be assigned randomly because some patients may receive optimal low doses, whereas others may receive high doses that might induce adverse effects. To address this issue, scholars demonstrated the up-and-down design (UDM) to estimate the median, whereas Derman demonstrated through nonparametric experimentation that dose levels could be centred around any given target quantile using the up-and-down designs using a biased coin.[3,4] Later, Durham *et al*.[5] generalised UDM as a biased coin design to estimate $ED_g$ using random walk rules. This method of the biased coin up-and-down design (BCUD) is used to assign doses sequentially by random walk rule in which efficacy or toxicity is assumed to be monotonically related to dose.

In dose-finding studies within the BCUD setting, the isotonic regression technique is utilised to estimate the effective dose for a particular drug ($ED_g$). Unlike simple linear regression, isotonic regression ensures that the regression function is monotonic, meaning it continuously increases or decreases. This method is suitable because it assumes that increasing the dose level increases the drug effect.[6] In this regression, the dose (x) serves as the predictor variable, whereas the response probability (P (x) = P (response | dose x)) acts as the dependent variable, representing the probability of a response to the drug.

This paper aims to describe these mathematical concepts using a diagrammatic explanation, specifically focusing on a section of the isotonic regression curve [Figure 1]. The paper also covers the estimation of naïve probability and adjusted probability using the pooled adjacent violators algorithm (PAVA) for the response.

The data used to elucidate the calculations needed to estimate $ED_{90}$ and its 95% confidence interval (CI) are given [Table 1 and Figure 2a] in annexure.

### Estimation of naive and PAVA probability

Table 2 shows the dose assigned to the participants (nDoses), number of patients (nTrials), number of successes (nEvents), the naïve probability and PAVA adjusted response rate at each distinctive dose level.

The naive probability is calculated [Table 2] using the following formula:

$$Naive\ \ Probability = f(\pi_i^*) = \frac{nEvents}{nTrials} \quad \text{(A)}$$

The PAVA probability is estimated using the PAVA algorithm for each dose level.[6] Under the PAVA algorithm, starting with the lowermost dose, we have to find the first adjoining pair of naive probability that violates the increasing ordering restriction (i.e. increasing dose level increases the drug effect), that is $f(\pi_i^*) \le f(\pi_{i+1}^*)$ where $f(\pi_i^*)\ \&\ f(\pi_{i+1}^*) \neq 0$. The PAVA probability for that pair of doses is

$$PAVA\ \ Probability = f(\pi_i) = f(\pi_{i+1}) = \frac{(nTrials_i * f(\pi_i^*)) + (nTrails_{i+1} * f(\pi_{i+1}^*))}{nTrials_i + nTrails_{i+1}} \quad \text{(B)}$$

In Figure 2b, the first adjoining pair of doses that violate the ordering restriction of naive probability is 7 and 8 μg, and the next successive pair of such doses is 9 and 10 μg, but for dose 10 μg, the naive probability is zero. Hence, the PAVA probability using equation (B) for doses 8 and 9 μg is

$$f(\pi)_{7\,\mu g} = f(\pi)_{8\,\mu g} = f(\pi)_{9\,\mu g} = f(\pi)_{10\,\mu g} = \frac{3*0.6667 + 4*0.7500}{3+4} = \mathbf{0.7143}$$

The same PAVA probability of 0.7143 is taken for preceding and succeeding doses of 7 and 10 μg [Table 2].

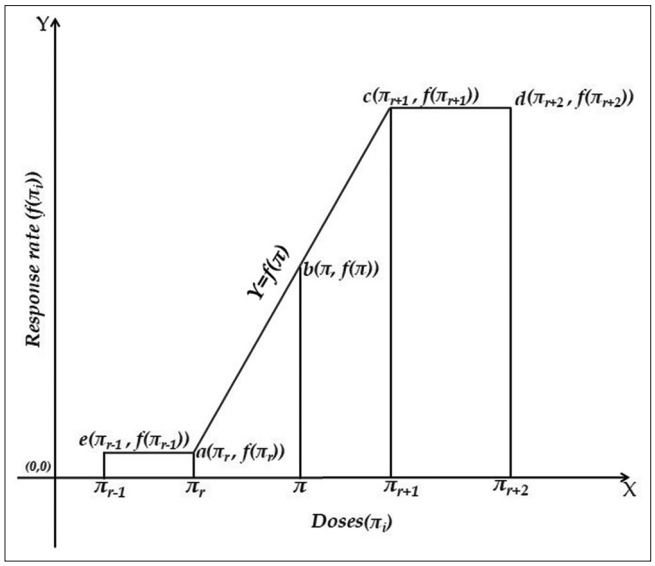


**Figure 1:** A small section of isotonic regression curve

## $ED_{90}$ and its 95% confidence interval

By substituting the values from Table 2 in annexure equation (8), the $ED_{90}$ is given below:

$$ED_{90} = 10 + \left(\frac{90}{100} - 0.7143\right) * \frac{11-10}{(0.9333-0.7143)} \approx \mathbf{10.848}$$

The precision of $ED_{90}$ is its 95% CI and is estimated using annexure equations (11) and (12):

$$Lower_p = \Phi(2 * 0.03969 - 1.96) = 0.03$$

$$Upper_p = \Phi(2 * 0.03969 + 1.96) = 0.9793$$

where, $\hat{z}_{adj} = \Phi^{-1}(0.51583) = 0.03969$

The final estimation of 95% bias-corrected bootstrap confidence interval (BCBCI) is estimated using the annexure equation (13):

$$\left[\widehat{ED}_{90_{LL}}, \widehat{ED}_{90_{UL}}\right] = \left[(3001 * 0.03)^{th}, (3001 * 0.9793)^{th}\right]$$

$$Boot\ estimate = \left(90^{th}, 2938^{th}\right) Boot\ estimate$$

Hence, the 95% CI for $ED_{90}$ is (9.25, 11.675) μg.

All calculations were performed using R 4.2.1 (R Foundation of Statistical Computing, Vienna, Austria), and the code used is given in Appendix I.

The bias of the original statistic ($ED_{90}$) = -0.076 is basically due to the discrete nature of the doses rather than the dimensional. The bias would be closer to zero if the dose ranges continuously, say, 10.1, 10.2, 10.3,…, 11.0, which would make the distance closer between the PAVA probability 0.7143 and 0.9000 for the dose of 10 μg.

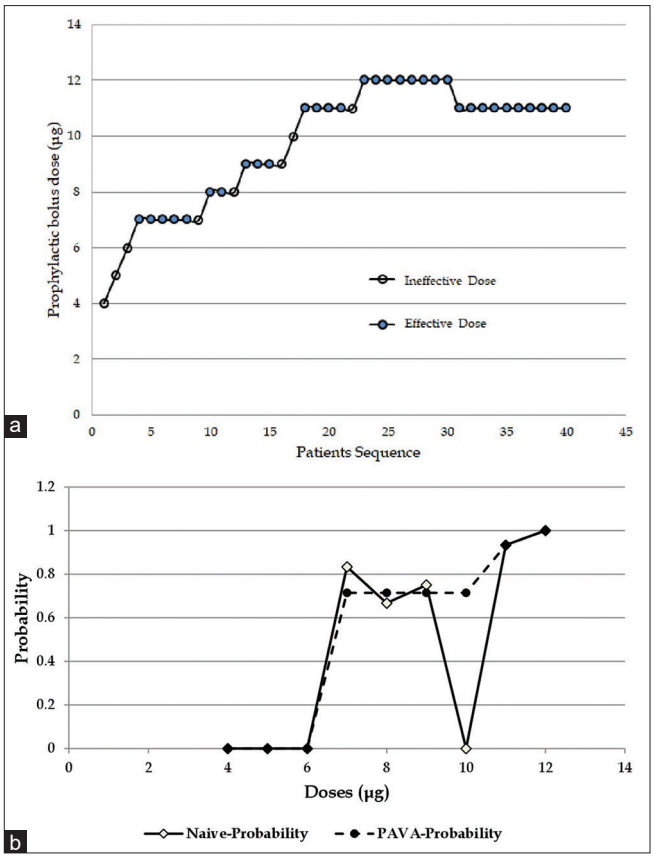


**Figure 2:** Plots showing the (a) patient's allotment sequence and the response to the assigned dose of norepinephrine prophylactic bolus (μg) and (b) naïve probability (observed response rate) and PAVA probability (adjusted response rate) for the assigned dose

## DISCUSSION

We explored the application of isotonic regression for estimating $ED_g$ along with its 95% confidence interval in the context of dose-finding studies. In anaesthesia, it is vital to assess how drug effects change with increasing doses using dose–response characterisation. In a general scenario, we may encounter a violation of the assumption of monotonicity in the observed probability, as occurred in the specified example. We employed the PAVA algorithm to rectify this violation and ensure the validity of the assumption.

The isotonic estimate of $ED_{90}$ in the BCUD setting has low bias and variance, particularly at low or high quantiles.[7] It also has a smaller mean square error than estimators from other methods.[8-10] We also assessed the overfitting of the estimate using bootstrapping, and the bias of the $ED_{90}$ estimate was very low [-0.076, Table 2].

**Table 1: Norepinephrine prophylactic bolus dose and the response of 40 successive women**

| Patient number | Norepinephrine Prophylactic bolus dose (μg) | Response[#] | Patient number | Norepinephrine Prophylactic bolus dose (μg) | Response[#] |
|---|---|---|---|---|---|
| 1 | 4 | F | 21 | 11 | S |
| 2 | 5 | F | 22 | 11 | F |
| 3 | 6 | F | 23 | 12 | S |
| 4 | 7 | S | 24 | 12 | S |
| 5 | 7 | S | 25 | 12 | S |
| 6 | 7 | S | 26 | 12 | S |
| 7 | 7 | S | 27 | 12 | S |
| 8 | 7 | S | 28 | 12 | S |
| 9 | 7 | F | 29 | 12 | S |
| 10 | 8 | S | 30 | 12 | S |
| 11 | 8 | S | 31 | 11 | S |
| 12 | 8 | F | 32 | 11 | S |
| 13 | 9 | S | 33 | 11 | S |
| 14 | 9 | S | 34 | 11 | S |
| 15 | 9 | S | 35 | 11 | S |
| 16 | 9 | F | 36 | 11 | S |
| 17 | 10 | F | 37 | 11 | S |
| 18 | 11 | S | 38 | 11 | S |
| 19 | 11 | S | 39 | 11 | S |
| 20 | 11 | S | 40 | 11 | S |

[#]*F* is failure, and S is success

**Table 2: Naive (observed) and PAVA probability and bootstrap estimates of 3000 boot replications**

| nDoses | nTrials | nSuccess | Naive probability | PAVA probability |
|---|---|---|---|---|
| 4 | 1 | 0 | 0 | 0 |
| 5 | 1 | 0 | 0 | 0 |
| 6 | 1 | 0 | 0 | 0 |
| 7 | 6 | 5 | 0.8333 | 0.7143 |
| 8 | 3 | 2 | $\pi_i^*$= 0.6667 | 0.7143 |
| 9 | 4 | 3 | $\pi_{i+1}^*$= 0.75 | 0.7143 |
| $\pi_r$=10 | 1 | 0 | 0 | $f(\pi_r)$=0.7143 |
| $\pi_{r+1}$=11 | 15 | 14 | 0.9333 | $f(\pi_{r+1})$=0.9333 |
| 12 | 8 | 8 | 1 | 1 |

| Statistic from bootstrapping (3000 replications) | Value |
|---|---|
| Original statistic ($ED_{90}$) | 10.848 |
| Mean of $ED_{90}$'s of Boot Replications | 10.772 |
| Median of $ED_{90}$'s of Boot Replications | 10.834 |
| Bias of Original statistic (ED90) (= Original Statistics – Mean of $ED_{90}$'s of Boot Replications) | -0.076 |
| Bias Correction: (No. of Boot Replicates <=Original Statistic)/(Total Replicates +1) | 0.51583 |
| Standard error of Boot Statistic (Mean of $ED_{90}$'s) | 0.626 |
| $\hat{z}_{adj}$ | 0.03969 |
| $z_{\alpha/2}$ | -1.96 |
| $z_{(1-\alpha/2)}$ | 1.96 |
| 2.5% Bias-corrected Lower Bound | 9.25 |
| 97.5% Bias-corrected Upper Bound | 11.675 |

To evaluate the precision of the estimated target dose, several methods estimate the bootstrap confidence interval with a confidence level of (1-α)*100%.[11] Simulation studies suggested that the BCBCI method, described in the paper, provides better balance, increased type I error and higher power than other bootstrap methods.[11,12]

Sequential dose-finding studies are appealing because they provide accurate and stable estimates with small sample sizes ranging from 20 to 40 patients.[5,8] These studies determine the critical intensity level (dose) at which a drug either produces or prevents a reaction in each patient. In such studies, the dose increment between the first dose at experimentation and the following subsequent doses is very small, and therefore, the outliers are unlikely.

The comparative analysis of the proposed technique against other methods is not included in this paper as it is beyond the scope of the paper and can be found elsewhere.[8] However, one notable comparison is the simplicity of the BCUD method compared to other methods; for example, the continual reassessment method requires a mathematical model to assign the dose and analyse previous dose responses for the next dose, which requires the involvement of a biostatistician.[13] On the other hand, the BCUD sequential method requires less computation and does not rely on a mathematical model to assign the doses. It also has simple statistical properties for estimating the target $ED_g$.[6] In contrast, other standard methods like logit and probit regression are complex and hence not widely used by anaesthesiologists. The isotonic estimate of $ED_g$, on the other hand,

does not demand technical expertise, and that is why anaesthesiologists should prefer this method to estimate any target dose without the help of the biostatistician.

## CONCLUSION

The isotonic regression adjusts the observed response rate (naive) using PAVA when it is not monotonically increasing with increasing dose levels. Also, it is straightforward to estimate the effective dose for a target quantile in the BCUD setting. Additionally, computer programming is needed only to estimate the 95% CI of $ED_g$.

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### Conflicts of interest

There are no conflicts of interest.

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**<H1>ANNEXURE [SUPPLEMANTARY MATERIAL AVAILABLE ONLINE]:**

**MATHEMATICAL CONCEPTS**

**Mathematical Illustration:**

Let $(\pi_1, f(\pi_1)),\ (\pi_2, f(\pi_2)),\ (\pi_3, f(\pi_3))\ \ldots\ldots\ldots (\pi_n, f(\pi_n))$ are the order set of doses and response rates, respectively.

Since isotonic regression seeks for a weighted least square fit, hence let $\varphi_i \geq 0$ (commonly $\varphi_i = 1$) be the weight assigned to each set of observation $(\pi_i, f(\pi_i))$ for all *i*.

In the isotonic regression, the observations are to be least deviated, which implies that the square of difference between the observed value and the predicted value should be minimal, that is $\left(f(\pi_i) - \widehat{f(\pi_i)}\right)^2 \approx 0\ \ for\ all\ i$ subject to the constraint that $\widehat{f(\pi_i)} \leq \widehat{f(\pi_j)}$ whenever $\pi_i \leq \pi_j$. The corresponding quadratic function (F) using the variables $\widehat{f(\pi_1)}, \widehat{f(\pi_2)}, \ldots\ldots, \widehat{f(\pi_n)}$ will be given by:

$$F = min \sum_{i=1}^{n} \varphi_i \left(f(\pi_i) - \widehat{f(\pi_i)}\right)^2 \qquad \ldots\ldots\ldots (1)$$

Subject to the constraint that $\widehat{f(\pi_i)} \leq \widehat{f(\pi_j)}$ for all $(i, j) \in \Theta$, where $\Theta = \{(i, j): \pi_i \leq \pi_j\}$

In the regular situation, the $f(\pi_i)$ values fall in an ordered set such as in $\Theta$, where $(i, j) = \{(i, i+1) : 1 \leq i < n\}$, but sometime $f(\pi_i)$ values may not fall in an ordered set, in such a scenario a simple iterative algorithm; that is, PAVA is used to maintain ordering restriction. Hence, in this case, PAVA algorithm can be used for solving the quadratic form given in equation (1).[14]

Let us consider a small section of isotonic regression curve [Figure 1] to generate its functional form.

In mathematics, we define slope as the rate of change in y-axis with respect to the change in x-axis of the line.

Let,

$\Delta f(\pi_i)$: Change in y-axis.

$\Delta \pi_i$: Change in x-axis.

So,

$$Slope = \frac{\Delta f(\pi_i)}{\Delta \pi_i} \qquad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Using the property of a straight line, we know that:

Slope of line **'ab'** = Slope of line **'ac'**

Using equation (2), we can write:

$$\frac{\Delta f(\pi_i)_{ab}}{\Delta(\pi_i)_{ab}} = \frac{\Delta f(\pi_i)_{ac}}{\Delta(\pi_i)_{ac}}$$

$$\Rightarrow \frac{f(\pi) - f(\pi_r)}{(\pi - \pi_r)} = \frac{f(\pi_{r+1}) - f(\pi_r)}{(\pi_{r+1} - \pi_r)}$$

$$\Rightarrow \boldsymbol{\pi = \pi_r + (f(\pi) - f(\pi_r)) * \frac{(\pi_{r+1} - \pi_r)}{(f(\pi_{r+1}) - f(\pi_r)}} \qquad \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Moreover, the slope of a line '**ea**' using equation (2) is given by

$$Slope\ of\ line\ 'ea' = \frac{\Delta f(\pi_i)_{ea}}{\Delta(\pi_i)_{ea}} = \frac{f(\pi_r) - f(\pi_{r-1})}{(\pi_r - \pi_{r-1})} \qquad \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

We know that the slope of a line parallel to x-axis is '0', and a line 'ea' is parallel to x-axis. Hence, from equation (4), we get

$$Slope\ of\ line\ 'ea' = 0 = \frac{f(\pi_r) - f(\pi_{r-1})}{(\pi_r - \pi_{r-1})}$$

$$0 = \frac{f(\pi_r) - f(\pi_{r-1})}{(\pi_r - \pi_{r-1})}$$

$$\boldsymbol{f(\pi_r) = f(\pi_{r-1})} \quad \text{..... (5)}$$

Similarly, for a line 'cd', we can get

$$\boldsymbol{f(\pi_{r+1}) = f(\pi_{r+2})} \quad \text{...... (6)}$$

Hence, using equations (3), (5) and (6), isotonic regression model yields a continuous piecewise linear function as mentioned below:

$$\boldsymbol{\pi} = \begin{cases} \boldsymbol{\pi_1} & \boldsymbol{if\ f(\pi) \le f(\pi_1)} \\ \boldsymbol{\pi_i + (f(\pi) - f(\pi_i)) * \frac{(\pi_{i+1} - \pi_i)}{(f(\pi_{i+1}) - f(\pi_i)}} & \boldsymbol{if\ f(\pi_i) \le f(\pi) \le f(\pi_{i+1})} \\ \boldsymbol{\pi_n} & \boldsymbol{if\ f(\pi) \ge f(\pi_n)} \end{cases}$$

…….. (7)

**Estimation of $ED_g$:**

Since the complete dose–response curve without any assumptions is generally estimated via linear interpolation between the point estimates, the isotonic regression estimate of $ED_g$ is the linearly interpolated dose between the adjusted proportion/rate of success just less than or equal to quantile 'g' $(f(\pi_r))$ and just greater than or equal to quantile 'g' $(f(\pi_{r+1}))$.[6,8]

Following the functional form of the isotonic regression model in equation (7), the interpolated dose between $f(\pi_r)$ and $f(\pi_{r+1})$ will be as follows:

$$\boldsymbol{\widehat{ED}_g = \pi_r + \left(\frac{g}{100} - f(\pi_r)\right) * \frac{(\pi_{r+1} - \pi_r)}{(f(\pi_{r+1}) - f(\pi_r)}}$$

…… (8)

where

$\widehat{ED}_g$= Interpolated Dose, $\pi_r = \max\left[\pi_i:\ f(\pi_i) \le \frac{g}{100}\right]$ and $\pi_{r+1} = \min\left[\pi_i:\ f(\pi_i) \ge \frac{g}{100}\right]$

The value of $f(\pi_i)$ is estimated using the PAVA algorithm, which is used in order to obtain an increased adjusted proportion/rate of $f(\pi) = \{f(\pi_1) \le f(\pi_2) \le \ldots.. \le f(\pi_n)\}$ based on observed rate $f(\pi^*)$ because sometimes it is possible that the observed proportion/rate

$f(\pi^*) = \{f(\pi_1^*), f(\pi_2^*), .., f(\pi_n^*)\}$ might not be increasing in nature with respect to increasing dose level, which is an implicit assumption for the dose-finding studies.[15-17]

To estimate $ED_g$, doses for the succeeding subsequent patients are adjusted using the BCUD sequential dose-finding method. In this method, the allocation scheme for determining $ED_g$, the probability of success $(\delta) = \frac{g}{100}$ and the target probability of changing the dose for successive patient = $(\beta) = (1-\delta)/\delta$.[5,6,18,19]

When a failure is observed, the dose is consistently increased for successive patients. In contrast, if success is observed, the successive patient will receive the preceding lower dose with the probability $(\beta)$ or will receive the same dose with the probability $(1 - \beta)$.

We randomly chose one of the published studies to elucidate the calculation of naïve and PAVA probability, estimation of $ED_{90}$ and its 95% CI and that study includes forty women who underwent caesarean delivery and were given the norepinephrine prophylactic bolus dose to maintain their systolic blood pressure (SBP).[15] The BCUD method is used to decide the dose level under the condition that the success is 'SBP of a woman is maintained above 80% of her baseline until delivery of the foetus'. The dose and response data of 40 successive women, extracted using the graph and PAVA table from the published study, are given in Table 1.

**Rules for PAVA probability if ordering restriction is violated:**

(i) If the estimated PAVA probability of a pair is less than the naive probability of the preceding dose and the naive probability of the succeeding dose is zero, then the PAVA probability for preceding and succeeding dose will also be same as the PAVA probability of that pair [refer Table 2].

(ii) Repeat the estimation of PAVA probability using equation (B) if any violation of increasing ordering restriction occurs further.

(iii) The PAVA probability is equal to naive probability for all those doses whose naive probability does not violate the increasing ordering restriction [refer Table 2 for doses 11 and 12 μg].

**Confidence Interval (CI):**

To estimate the 95% CI of $ED_g$, the bias-corrected bootstrap (BCB) method using 3000 bootstrap replications of $\widehat{ED}_g$ is used.[5,18-20] Each replication is obtained by extracting bootstrap data of appropriate sample size and the BCUD method, considering that the true dose–response rate at every dose is $f(\pi_i), i = 1, 2, 3, \dots\dots, n$, estimated based on the original data.

To estimate (1 - α)*100% BCBCI, we need to calculate the z-scores corresponding to the percentiles of the confidence interval's lower and upper limits using the following equations[11,21]:

$$\boldsymbol{Lower_{z-score}: 2 * \hat{z}_{adj} + z_{\alpha/2}} \quad \ldots\ldots\ldots (9)$$

$$\boldsymbol{Upper_{z-score}: 2 * \hat{z}_{adj} + z_{(1-\alpha/2)}} \quad \ldots\ldots\ldots (10)$$

where $\boldsymbol{\hat{z}_{adj} = \Phi^{-1}\left(\frac{No.\ of\ Bootstrap\ estimate \leq ED_g}{B+1}\right) = \Phi^{-1}(Bias\ correction)}$. If this probability is equal to 0.50, then the estimated $ED_g$ from sample data is same as the median of the bootstrap sampling distribution, and hence, the bias term $\boldsymbol{2 * \hat{z}_{adj}}$ will be equal to zero. The terms $\Phi^{-1}$ and B are the inverse normal cumulative distribution function and the number of bootstrap replications, respectively. $z_{\alpha/2}\ and\ z_{1-\alpha/2}$ are the z-scores corresponding to the $\frac{\alpha}{2} \times 100^{th}$ and $(1-\frac{\alpha}{2}) \times 100^{th}$ percentile of the standard normal distribution, respectively. $\hat{z}_{adj}$ is a measure of the median bias of the sample and bootstrap estimators, and hence, $\hat{z}_{adj}$ value is multiplied by 2.

The proportions corresponding to the $lower_{z\text{-score}}$ and $upper_{z\text{-score}}$ in equations 9 and 10 can be obtained using the following equations:

$$\boldsymbol{Lower_p: \Phi\left(2 * \hat{z}_{adj} + z_{\alpha/2}\right)} \quad \ldots\ldots\ldots (11)$$

$$\boldsymbol{Upper_p: \Phi\left(2 * \hat{z}_{adj} + z_{(1-\alpha/2)}\right)} \quad \ldots\ldots\ldots (12)$$

where Φ = normal cumulative distribution function.

The bias-corrected $100(1-\alpha)\%$ CI for $ED_{90}$ after sorting the boot replicates in increasing order is given by:

$$\left[\widehat{ED}_{g_{LL}}, \widehat{ED}_{g_{UL}}\right] = \left[\left((B+1) * Lower_p\right)^{th}, \left((B+1) * Upper_p\right)^{th}\right] Boot\ estimate \quad \ldots\ldots\ldots (13)$$

## Appendix I: R-Code

| Code | Comments |
|---|---|
| library(boot) | **# Open boot library** |
| library(ed50simulation) | **# Open ed50simulation library** |
| doseSequence<-<br>c(4,5,6,7,7,7,7,7,7,8,8,8,9,9,9,9,10,11,11,11,11,11,12,12,12,12,12,12,12,12,11,11,11,11,11,11,11,11,11,11) | **# Store dose sequence** |

```
responseSequence<-
c(0,0,0,1,1,1,1,1,1,0,1,1,0,1,1,1,0,0,1,1,1,1,0,1,1,1,1,1,1,1,1,1,1,
1,1,1,1,1,1,1,1,1)
dF <- data.frame(doseSequence, responseSequence)

pavaData <- preparePava(dF)

set.seed(sample(size = 1,x=seq(1000)))

bootResult <- boot(data = dF,
                   statistic = bootIsotonicRegression,
                   R = 3000,
                   sim = 'parametric',
                   ran.gen = bootIsotonicResample,
                   mle = list(baselinePava = pavaData,
                              firstDose = doseSequence[1],
                              PROBABILITY.GAMMA = 0.9),
                   baselinePava = pavaData,
                   PROBABILITY.GAMMA = 0.9)

prediction <- bootBC.ci(tObserved = bootResult$t0[3],
                        tBoot = bootResult$t[, 3],
                        conf = 0.95)
```

**# Store response sequence**

**# Create data frame using dose and response sequence**

**# Calculates naive and PAVA probability**

**# Seeding the boot replicates so that the output will be same on each compilation.**

**# Generate the 3000 bootstrap replicates and bootstrap results to compute effective dose for 90 %( 0.9) of the patients.**

**# To compute the estimate of $ED_g$ and 95% CI of it using the boot results.**