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Assessing departure from dose linearity under a repeated measures incomplete block design

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Dose proportionality/linearity is a desirable property in pharmacokinetic studies. Various methods have been proposed for its assessment. When dose proportionality is not established, it is of interest to evaluate the degree of departure from dose linearity. In this paper, we propose a measure of departure from dose linearity and derive an asymptotic test under a repeated measures incomplete block design using a slope approach. Simulation studies show that the proposed method has a satisfactory small sample performance in terms of size and power. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: dose response study; pharmacokinetic parameters; Mahalanobis distance; heteroscedastic variance; sample size calculation.

1. INTRODUCTION

In pharmaceutical research, pharmacokinetic (PK) studies are often conducted not only to assess drug tolerance and safety in phase I clinical development but also to characterize drug response curve with respect to efficacy in phase II clinical development. Rodda *et al.* [1] indicated that the clinical investigation of the dose response curve in terms of some PK parameters such as area under the blood or plasma concentration time curve (AUC) and maximum concentration (C_{max}) can be classified into categories of (i) dose ranging study, which estimates the minimum effective dose and maximum tolerable dose, (ii) dose response existence, and (iii) dose response characterization, which describes the shape of the dose response curves and decides whether there is a clinically meaningful increase in response between the minimum effective and maximum tolerable doses. Ruberg [2,3] provided a comprehensive review of design, analysis, and interpretations of dose response studies. For a broader and thorough discussion of statistical issues in dose findings, see Ting [4] and Ting and Grieve [5].

Among various types of dose response relationships, dose proportionality is probably the most desirable dose response relationship between doses and PK responses such as AUC due to its easy and understandable interpretation that if we double the dose, we expect the AUC to be doubled. Under the property of dose proportionality or linearity, PK responses can be easily predicted with various dose levels. Dose proportionality/linearity is often assessed based on a $J \times L$ repeated measures incomplete block design under which study subjects are equally randomized to one of the J dose sequences with each subject receiving different doses at L dosing periods. As an illustration, we consider a dose response study with four doses (60, 120, 240, and 480 mg) under a 3×3 unbalanced incomplete block design with three dose sequences: 60–120–480 mg, 60–240–480 mg, and 60–120–240 mg. This example will be examined later in the paper.

Several methods have been proposed for assessing dose proportionaility/linearity under various designs [6]. Chow and Liu [6] suggested a slope approach for assessing dose proportionality/linearity. Specifically, let μ_i be the mean dose response at the ith dose d_i, $i = 1,\ldots,I$, where $d_1 < \cdots < d_I$. Define the adjacent slope $\theta_i = (\mu_{i+1}-\mu_i)/(d_{i+1}-d_i)$ for $i = 1,\ldots,I-1$, and the difference of the adjacent slopes $\phi_i = \theta_{i+1}-\theta_i$, for $i = 1,\ldots,I-2$. Then, dose proportionality/linearity implies that ϕ_is are all 0. In practice, as pointed out by Chow and Liu [6] and Law and Chow [7], when there is departure from dose linearity (i.e. the ϕ_is are not all 0), it is of clinical importance to assess the extent of such departure from linearity. In practice, deviation (or departure) from the established dose proportionality/linearity is expected, which may have a drastic impact on clinical responses (outcomes).

In this paper, we propose a method to assess the departure from dose linearity based on ϕ_is, the differences of the adjacent slopes of the dose response curve. The paper is organized as follows. In next section, a measure of departure from dose linearity is suggested under a $J \times L$ repeated measures incomplete block design. Also included in this section are the hypotheses of minor departure from dose linearity. An asymptotic test is proposed and a formula for sample size calculation based on a pre-study power analysis is derived in Section 3. Although it is based on hypothesis testing procedures, our proposed method can also be applied in estimating the departure from linearity, which is also given in Section 3. In Section 4, a real example concerning a dose proportionality/linearity study under a 3×3 unbalanced incomplete block design is discussed and simulations are performed to evaluate the small sample performance of the proposed test. Some discussions are provided in the last section.

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2. MODELS AND HYPOTHESES

Consider a dose response study involving I dose levels $d_1 < \cdots < d_I$, where I is assumed to be fixed. Consider a design in which subjects are randomized to one of the J dose sequences and each subject receives different doses at L dosing periods. The number of dosing periods L is chosen to be smaller than I, the number of doses in consideration. In addition, we require that each dose appear in at least one dose sequence but no more than once within any such dose sequence. This results in an incomplete block design with J blocks of block size L. According to Westlake [8], in a clinical setting, an incomplete block design has the advantages that (i) it reduces the number of required washout periods, (ii) it requires fewer blood samples, and (iii) it may result in fewer patient dropouts. Alternatively, such a design can be viewed as a special crossover design as each patient receives more than one dose.

Let y_{ijk} be the dose response of subject k who receives dose i in dose sequence j, $i = 1,\ldots,I$, $j = 1,\ldots,J$, $k = 1,\ldots,n$. By design, y_{ijk} is defined only when dose i appear in dose sequence j. We consider the following repeated measurement model:

$$y_{ijk} = \mu_i + \alpha_{ij} + d_i(e_{jk} + \varepsilon_{ijk}) \tag{1}$$

where $\sum_{j=1}^{J} \alpha_{ij} = 0$ for $i = 1, \ldots, I$, $d_i e_{jk}$ represents the random effect of the subject k nested in sequence j, and $d_i\varepsilon_{ijk}$ represents the random error. We assume that e_{jk}s are independent, identically distributed (i.i.d.) as a normal random variable with mean 0 and variance σ_s^2, i.e. $N(0, \sigma_s^2)$, and ε_{ijk}s are i.i.d. normally distributed as $N(0, \sigma_e^2)$, and e_{jk}s and ε_{ijk}s are mutually independent. It is important to know that α_{ij} is defined only when dose i appears in sequence j, and thus there are a total of $J \times L$ of such parameters. The constraint $\sum_{j=1}^{J} \alpha_{ij} = 0$ for any fixed i implies that for all the sequences that involve treatment i, the sum of the corresponding sequence effects must be 0. This constraint ensures that the parameters μ_i and α_{ij} are all estimable as long as dose i appears in some dose sequence(s), which is automatically satisfied by the design. Model (1) is similar to the null-carryover-effect models considered by Chinchilli and Esinhart [9] except that it has a heteroscedastic variance structure, that is, the standard deviation of the dose response y_{ijk} is proportional to the dose d_i. For more discussions regarding the theory and applications of crossover designs, see Senn [10], Vonesh and Chinchilli [11], Chow and Liu [12], and Jones and Kenward [13].

As discussed in Section 1, dose proportionality implies that $\phi_i = 0$ for $i = 1,\ldots, I$–2. In general, ϕ_i can be considered as measuring the local nonlinearity of the dose response curve around dose level i. To estimate ϕ_i's, we express ϕ_i as a function of μ_i's. Specifically, let $\boldsymbol{\mu} = (\mu_1,\ldots,\mu_I)'$, $\boldsymbol{\phi} = (\phi_1,\ldots,\phi_{I-2})'$, and define an $(I-2) \times I$ matrix

$$\mathbf{M} = \begin{pmatrix} \frac{1}{d_2 - d_1} & \frac{d_1 - d_3}{(d_2 - d_1)(d_3 - d_2)} & \frac{1}{d_3 - d_2} & & \\ & \frac{1}{d_3 - d_2} & \frac{d_2 - d_4}{(d_3 - d_2)(d_4 - d_3)} & \frac{1}{d_4 - d_3} & \\ & & \ddots & & \\ & & & \frac{1}{d_{I-1} - d_{I-2}} & \frac{d_{I-2} - d_I}{(d_{I-1} - d_{I-2})(d_I - d_{I-1})} \quad \frac{1}{d_I - d_{I-1}} \end{pmatrix}$$

then $\boldsymbol{\phi} = \mathbf{M}\boldsymbol{\mu}$.

We first obtain an estimator of μ_i. Let $m_{ii'}$ be the number of times that both doses i and i' appear in the same dose sequence and when $i = i'$, m_{ii} is understood as the number of times that dose i appears in the J dose sequences. The integers $m_{ii'}$'s are completely determined by the design and do not depend on n, the number of subjects per dose sequence. Let $\bar{y}_{ij\cdot} = \sum_{k=1}^{n} y_{ijk}/n$, then $\hat{\mu}_i = \sum_{j=1}^{J} \bar{y}_{ij\cdot}/m_{ii}$, and it can be shown that $\hat{\boldsymbol{\mu}} = (\hat{\mu}_1,\ldots,\hat{\mu}_I)'$ is an unbiased estimator of μ with covariance matrix

$$\sum = n^{-1}[\mathbf{D}\boldsymbol{\Lambda}_1\mathbf{D}\sigma_s^2 + \mathbf{D}\boldsymbol{\Lambda}_2\mathbf{D}\sigma_e^2] \tag{2}$$

where $\mathbf{D}$ is an $I \times I$ diagonal matrix whose ith diagonal entry is d_i, $\boldsymbol{\Lambda}_1$ is an $I \times I$ matrix whose (i,i')th entry is $m_{ii'}/(m_{ii}m_{i'i'})$, and $\boldsymbol{\Lambda}_2$ is an $I \times I$ diagonal matrix whose ith diagonal entry is m_{ii}^{-1}. Note that by design and the constraint $\sum_{j=1}^{J} \alpha_{ij} = 0$, $\hat{\boldsymbol{\mu}}$ is estimable, hence its covariance matrix $\boldsymbol{\Sigma}$ is positive definite. Consequently, ϕ_i can be estimated by $\hat{\phi}_i = (\hat{\mu}_{i+2} - \hat{\mu}_{i+1}/d_{i+2} - d_{i+1}) - (\hat{\mu}_{i+1} - \hat{\mu}_i/d_{i+1} - d_i)$. Alternatively, in matrix form, parameter $\boldsymbol{\phi}$ can be estimated by $\hat{\boldsymbol{\phi}} = \mathbf{M}\hat{\boldsymbol{\mu}}$, which has a multivariate normal distribution with mean $\boldsymbol{\phi}$ and variance $\mathbf{M}\boldsymbol{\Sigma}\mathbf{M}'$. Therefore, $\hat{\boldsymbol{\phi}}'(\mathbf{M}\boldsymbol{\Sigma}\mathbf{M}')^{-1}\hat{\phi}$ has a noncentral chi-squared distribution with I–2 degrees of freedom and noncentrality parameter $n\lambda$, denoted as $\chi^2_{I-2}(n\lambda)$, where

$$\lambda = \boldsymbol{\phi}'[\mathbf{M}(\mathbf{D}\boldsymbol{\Lambda}_1\mathbf{D}\sigma_s^2 + \mathbf{D}\boldsymbol{\Lambda}_2\mathbf{D}\sigma_e^2)\mathbf{M}']^{-1}\boldsymbol{\phi}. \tag{3}$$

For a given design, matrices $\mathbf{M}$, $\mathbf{D}$, $\boldsymbol{\Lambda}_1$, and $\boldsymbol{\Lambda}_2$ are all known, thus the parameter λ is a function of $\boldsymbol{\phi}$ and the variance parameters σ_s^2 and σ_e^2. For fixed σ_s^2 and σ_e^2, λ is a positive definite quadratic form in ϕ_is, and hence can assume any positive value, depending on the choice of ϕ_is. The parameter λ can be regarded as a squared ''distance'' between $\boldsymbol{\phi}$ and $\mathbf{0}$. Precisely, $\lambda = 0$ implies $\boldsymbol{\phi} = \mathbf{0}$, i.e. dose linearity, and a large value of λ indicates a serious departure from dose linearity. On the other hand, when $\boldsymbol{\phi}$ is fixed, λ is a decreasing function of σ_s^2 and σ_e^2, indicating good sensitivity to random variations. In fact, λ is closely related to the squared Mahalanobis distance used in cluster analysis. Therefore, we choose λ as a global measure of severity of departure from dose linearity. Although dose proportionality is the most desirable property, the power of testing such an alternative hypothesis is equal to the size α since the alternative space contains only one single parameter $\lambda = 0$. On the other hand, we believe strict dose proportionality is rare and for many practical purpose, it would suffice if we could confirm that the dose response is 'very close to' proportionality/linearity, or equivalently, the departure from linearity is ''minor''. Therefore, we consider assessing the following hypotheses for a minor departure from dose linearity

$$H_0 : \lambda \geqslant \lambda_0, \quad \text{versus} \quad H_1 : \lambda < \lambda_0 \tag{4}$$

where λ_0 is a pre-specified clinically relevant limit. The choice of λ_0 will be further discussed in Sections 4 and 5. We set minor departure as the alternative because that is the property that we are eager to establish.

3. MINOR DEPARTURE FROM DOSE LINEARITY

3.1. Testing method

To derive a test for (4), we need to estimate the variance components σ_s^2 and σ_e^2. Let $v_{ij}=(\mu_i+\alpha_{ij})/d_i$, then $\mu_{ijk}=y_{ijk}/d_i$ satisfies model

$$u_{ijk} = v_{ij}+e_{jk}+\varepsilon_{ijk}$$

It can be shown that

$$\widehat{\sigma_s^2+\sigma_e^2} = \sum_{j=1}^{J}\sum_{i=1}^{I}\sum_{k=1}^{n}(u_{ijk}-\bar{u}_{ij.})^2/[JL(n-1)] \tag{5}$$

and

$$\hat{\sigma}_s^2 = \sum_{i\neq i',m_{ii'}>0}\sum_{j=1}^{J}\sum_{k=1}^{n}(u_{ijk}-\bar{u}_{ij.})(u_{i'jk}-\bar{u}_{i'j.})/ [JL(L-1)(n-1)] \tag{6}$$

are unbiased estimators of $\sigma_s^2+\sigma_e^2$ and σ_e^2, respectively. Let $\hat{\boldsymbol{\Sigma}}$ be the estimator of $\boldsymbol{\Sigma}$ with σ_s^2 and σ_e^2 replaced by $\hat{\sigma}_s^2$ and $\widehat{\sigma_s^2+\sigma_e^2}-\hat{\sigma}_s^2$ defined in (5) and (6), respectively, and define

$$T = \hat{\boldsymbol{\mu}}'\mathbf{M}'(\mathbf{M}\hat{\boldsymbol{\Sigma}}\mathbf{M}')^{-1}\mathbf{M}\hat{\boldsymbol{\mu}}' \tag{7}$$

Theorem 1. Assume model (1) and let T be defined as in (7), then T is asymptotically distributed as $\chi^2_{I-2}(n\lambda)$ as $n\to\infty$. Thus, an asymptotic size α test rejects H_0 in (4) if and only if $T<\chi^2_{I-2,1-\alpha}(n\lambda_0)$, where $\chi^2_{m,\alpha}(\xi)$ is the $(1-\alpha)$th percentile of a noncentral chi-square random variable with noncentrality parameter ξ and m degrees of freedom.

Proof: Since $\hat{\boldsymbol{\mu}}$ has a multivariate normal distribution with mean $\boldsymbol{\mu}$ and covariance matrix Σ, then $\hat{\boldsymbol{\phi}}=\mathbf{M}\hat{\boldsymbol{\mu}}$ has a multivariate normal distribution with mean $\boldsymbol{\phi}$ and covariance matrix $\mathbf{M}\boldsymbol{\Sigma}\mathbf{M}'$. Therefore, $\hat{\boldsymbol{\mu}}'\mathbf{M}'(\mathbf{M}\boldsymbol{\Sigma}\mathbf{M}')^{-1}\mathbf{M}\hat{\boldsymbol{\mu}}$ has a noncentral chi-square distribution with $I2$ (the rank of $\mathbf{M}$) degrees of freedom and noncentrality parameter $n\lambda$. Since $\hat{\boldsymbol{\Sigma}}$ is a consistent estimator of $\boldsymbol{\Sigma}$ as $n\to\infty$, T defined in (7) has an asymptotic noncentral chi-square distribution. The result follows from the fact that T is asymptotically distributed as $\chi^2_{I-2}(n\lambda)$, and the function $P(T<t)$ is a decreasing function of λ for any fixed t. Hence, $sup_{\lambda\geqslant\lambda_0}P_\lambda\times(T<t)=P_{\lambda_0}(T<t)=\alpha$ when $t=\chi^2_{I-2,1-\alpha}(n\lambda_0)$. This completes the proof.

When the sample size n per dose sequence is small, it is well known that $\chi^2_{I-2}(n\lambda)$ is not a good approximation of the distribution of T. By mimicking the Hotelling's T^2 distribution, we suggest using $(J(I-2)(n-1)/J(n-1)-I+3)F_{I-2,J(n-1)-I+3}(n\lambda)$ to approximate the distribution of T. As the result, when n is small, we reject H_0 in (4) if and only if $T<(J(I-2)(n-1)/J(n-1)-I+3)F_{I-2,J(n-1)-I+3,1-\alpha}(n\lambda)$, where $F_{a,b,\alpha}(\xi)$ denotes the (1α)th percentile of the noncentral F-distribution with degrees of freedom (a, b) and noncentrality parameter ξ. The small sample distribution of T may also be approximated by a noncentral F-distribution based on a Kenward-Roger type method [14] but the determination of the denominator degrees of freedom is computationally involved.

From Theorem 1, it is seen that the size and the power of the proposed test are univariate functions of λ. Although handling univariate functions is mathematically convenient, it is of practical advantage to view the size and the power as functions of the nuisance parameters ϕ_is, σ_e^2, σ_s^2, and design parameters as these parameters have clear clinical interpretations. On the other hand, it is true that since λ is a smooth deterministic function of ϕ_i's, σ_e^2, σ_s^2, and design parameters, the size and the power of the proposed test will change smoothly as these nuisance parameters vary smoothly.

3.2. Sample size calculation

Sample size calculation can be obtained based on a pre-study power analysis for achieving a desired power of $1-\beta$. We propose the following formula for sample size calculation.

Theorem 2. Consider testing hypotheses in (4) under model (1). To achieve a desired power of $1-\beta$ at a given alternative value λ at the α level of significance, the number of subjects n required for each dose sequence is given by

$$n = max\left\{4\left(z_\beta\sqrt{\lambda}+z_\alpha\sqrt{\lambda_0}\right)^2/(\lambda_0-\lambda)^2, 2\right\} \tag{8}$$

Proof: Under a given alternative $\lambda<\lambda_0$ in (4), the test statistic T in (7) is distributed as a noncentral chi-square random variable with noncentrality parameter $n\lambda$ and $I-2$ degrees of freedom. As indicated in Johnson *et al.* [15], when n is large, the noncentral chi-square distribution $\chi^2_{I-2}(n\lambda)$ can be approximated by a normal distribution as follows:

$$\chi^2_{I-2}(n\lambda) = N(I-2+n\lambda, 2I-4+4n\lambda)+O_p(n^{-1/2}) \tag{9}$$

By (9), the following equation:

$$P(\chi^2_{I-2}(n\lambda)<\chi^2_{I-2,1-\alpha}(n\lambda_0)) = 1-\beta$$

is asymptotically equivalent to

$$\frac{n(\lambda_0-\lambda)-z_\alpha\sqrt{2(I-2)+4n\lambda_0}}{\sqrt{2(I-2)+4n\lambda}} = z_\beta$$

or approximately (as $n\to\infty$),

$$\frac{\sqrt{n}(\lambda_0-\lambda)}{2\sqrt{\lambda}}-z_\alpha\sqrt{\frac{\lambda_0}{\lambda}} = z_\beta$$

The result follows after solving the above equation and realizing the constraint that $n\geqslant 2$ to ensure that σ_s^2 and σ_e^2 are estimable.

We make some remarks on formula (8). First, it is easily verified that n is an increasing function of λ over the alternative space $[0, \lambda_0]$ in (4). Therefore, by setting $\lambda=0$, which corresponding to dose linearity, we obtain the minimal sample size as $n_{\min}=max\{4z_\alpha^2/\lambda_0, 2\}$. On the other hand, when λ is close to the threshold value λ_0, the sample size required is approximately $4\lambda_0(z_\beta+z_\alpha)^2/(\lambda-\lambda_0)^2$, hence the increase in sample size would be drastic. Second, intuitively the choice of design parameters J and L may have a direct impact on the sample size. However, we see that λ, hence the desired sample size n, depends on J and L only through the design configuration parameters $m_{ii'}$s, which cannot be uniquely determined by J and L. Therefore, selection on J and L alone generally will not yield a design with optimal sample size. On the other hand, it would be interesting, yet challenging, to find the optimal $m_{ii'}$'s when J and L are pre-fixed based on either economic or administrative consideration.

3.3. Departure from dose linearity as an estimation problem

Our proposed method is based on hypothesis testing procedures. Conducting such a procedure entails a choice of the threshold value λ_0. Some guidance on the choice of λ_0 will be provided in Section 4. In reality, it is hard, if not impossible, to make an objective selection of λ_0. One way to avoid the selection of λ_0 is to treat the problem of assessing the departure from dose linearity as an estimation problem as is suggested by one of the referees.

The local departure from dose linearity at dose d_i is characterized by ϕ_i, the difference in the adjacent slopes, $i = 1,\ldots,I-2$. In Section 3.1, $\boldsymbol{\phi}$, the vector of the local departures, is estimated as $\mathbf{M}\hat{\mu}$. Then the asymptotic $100\times(1-\alpha)\%$ confidence region is given by $\{\boldsymbol{\phi} : (\hat{\boldsymbol{\phi}}-\boldsymbol{\phi})'(\mathbf{M}\hat{\boldsymbol{\Sigma}}\mathbf{M})^{-1}(\hat{\boldsymbol{\phi}}-\boldsymbol{\phi}) \leqslant \chi^2_{I-2,\alpha}\}$, where $\chi^2_{m,\alpha}$ is the $(1-\alpha)$th percentile of a chi-square random variable with m degrees of freedom.

As is mentioned in Section 2, the global departure measure λ is a squared distance between $\boldsymbol{\phi}$ and $\mathbf{0}$. Since the test statistic T in (7) is asymptotically distributed as $\chi^2_{I-2}(n\lambda)$, we obtain an asymptotically unbiased estimate of λ as $\hat{\lambda} = max\{(T/n) - ((I-2)/n), 0\}$. Define

$$\hat{\lambda}_L = max\left\{\hat{\lambda} - z_{\alpha/2}\sqrt{\frac{4\hat{\lambda}}{n}+\frac{2I-4}{n^2}}, 0\right\},$$
$$\hat{\lambda}_U = \hat{\lambda}+z_{\alpha/2}\sqrt{\frac{4\hat{\lambda}}{n}+\frac{2I-4}{n^2}} \quad (10)$$

then by (9), $[\hat{\lambda}_L, \hat{\lambda}_U]$ is an asymptotical $100\times(1-\alpha)\%$ confidence for λ, where z_α denotes the $(1-\alpha)$th percentile of standard normal distribution. Using the small sample approximation suggested at the end of Section 3.1, an asymptotically unbiased estimator with small sample correction is given by $\tilde{\lambda} = max\{\big(1-((I-1)/J(n-1))\big)T/n - ((I-2)/n), 0\}$. Define $\tilde{\lambda}_L$ and $\tilde{\lambda}_U$ by replacing $\hat{\lambda}$ with $\tilde{\lambda}$ in the expressions of $\hat{\lambda}_L$ and $\hat{\lambda}_U$ in (10), then $[\tilde{\lambda}_L, \tilde{\lambda}_U]$ is an asymptotical $100\times(1-\alpha)\%$ confidence interval for λ with small sample correction.

4. NUMERICAL STUDIES

4.1. An example

4.1.1. Design and data

To illustrate the proposed testing procedure for assessing minor departure from linearity, we consider a dose response study conducted on 18 subjects for evaluation of dose linearity of a pharmaceutical compound. The study involved four dose levels: 60, 120, 240, and 480 mg. There were threes dose sequences and each dose sequence consisted of three different doses. Thus, $I=4$, $J=3$, and $L=3$. The 18 subjects were randomized to the three dose sequences with six subjects in each dose sequence (i.e. $n=6$). Within a given dose sequence, AUC data were obtained at three different dosing periods for every subject. The AUC data are given in Table I.

The mean dose responses at the four dose levels are estimated as $\hat{\boldsymbol{\mu}} = (190.57, 459.73, 1297.75, 3400.98)'$ with the corresponding differences in the adjacent slopes estimated as $\hat{\phi}_1 = 2.49, \hat{\phi}_2 = 1.78$. The variance components σ^2_s and σ^2_e are estimated as $\hat{\sigma}^2_s = 5.12$ and $\hat{\sigma}^2_e = 0.69$, respectively. The plot of dose response profiles for the three dose sequences together with the mean dose responses profile (Figure 1) suggest that the departure from linearity might not be minor since the mean response at 480 mg is more than double than the expected mean responses if the dose proportional holds.

Table I. AUC data.

Sequence	Subject ID	Dose levels 60 mg	120 mg	240 mg	480 mg
1	1	35.25	227.95		2797.65
1	4	70.50	268.30		2738.00
1	8	412.05	911.90		5967.25
1	11	49.85	218.60		1714.10
1	15	334.60	717.00		4777.30
1	18	439.00	839.85		4354.10
2	2	63.45		990.70	2649.60
2	6	207.35		1229.65	3110.15
2	9	99.70		829.80	2207.95
2	12	280.30		2144.20	4332.15
2	13	268.70		1690.15	4217.55
2	16	130.55		605.85	1945.90
3	3	213.85	529.90	1302.15	
3	5	285.95	717.60	2318.40	
3	7	66.20	111.50	862.50	
3	10	105.20	321.55	780.65	
3	14	74.45	301.10	1249.60	
3	17	293.35	351.50	1569.30	

4.1.2. Choosing λ_0

To test the hypotheses (4), we must choose a λ_0. Since λ is a global measure of nonlinearity, it is reasonable to compute λ for a collection of common dose response patterns and choose λ_0 such that the departure from linearity in the true dose response is no more serious as some of the common response patterns. For the given example, we propose to consider the following four types of dose response curves whose plots are shown in Figure 2:

$$\text{linear dose curve}: \quad \text{response} = 7.08\ \text{dose} \quad (11)$$

$$\text{square root dose curve}: \quad \text{response} = 155.19\sqrt{\text{dose}} \quad (12)$$

$$\text{quadratic dose curve}: \quad \text{response} = 0.015\ \text{dose}^2 \quad (13)$$

$$\text{logistic dose curve}: \quad \text{response} = 3400\left[1+exp\{-(\text{dose}-240)/35\}\right]^{-1} \quad (14)$$

The above dose response curves are chosen such that they all pass through the origin and approximately the point (480, 3400), where 3400 is the estimated mean AUC at the highest dose 480 mg from the example data. From Table I, we have $(\hat{\sigma}_s, \hat{\sigma}_e) = (2.26, 0.83)$, the estimated values based on the example data. We then compute the value λ as 0.00, 1.22, 4.60, 7.03 for the dose patterns (11) through (14), respectively. We *may* choose $\lambda_0 = 1.22$, if we view the departure from linearity in the square root dose response curve in Figure 2 as minor. In practice, such a decision should be made in collaboration with clinical scientists.

4.1.3. Illustration of the procedure

We illustrate the proposed testing procedure with $\lambda_0 = 1.22$, selected empirically in the previous section. The covariance matrix of $\hat{\boldsymbol{\mu}}$ is estimated as

$$\hat{\boldsymbol{\Sigma}} = \begin{pmatrix} 1162.03 & 2048.26 & 4096.53 & 8193.06 \\ 2048.26 & 6972.17 & 6144.79 & 12\,289.59 \\ 4096.53 & 6144.79 & 27\,888.67 & 24\,579.17 \\ 8193.06 & 12\,289.59 & 24\,579.17 & 111\,554.67 \end{pmatrix}$$

and the test statistic is $T = 6.27$. Using the small sample version of the test described at the end of Section 3.1, we fail to reject the null hypothesis in (4) since $T = 6.27 > (30/14) F_{2,14,0.95}(6 \times 1.22) = 1.83$, implying that there is no statistical evidence to conclude that the departure from linearity is minor.

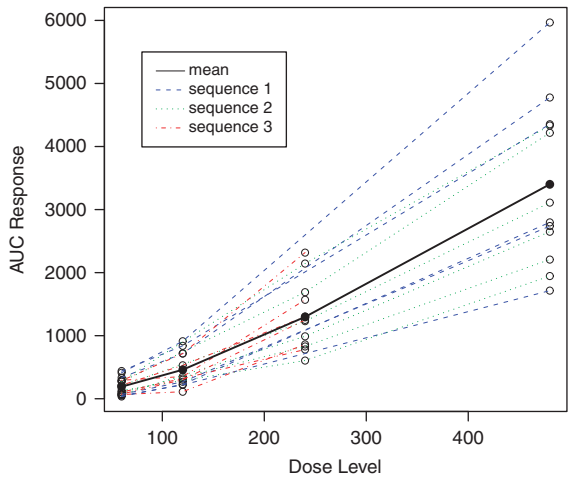

Figure 1. Plot of dose response profiles. Solid dots represent the mean responses.

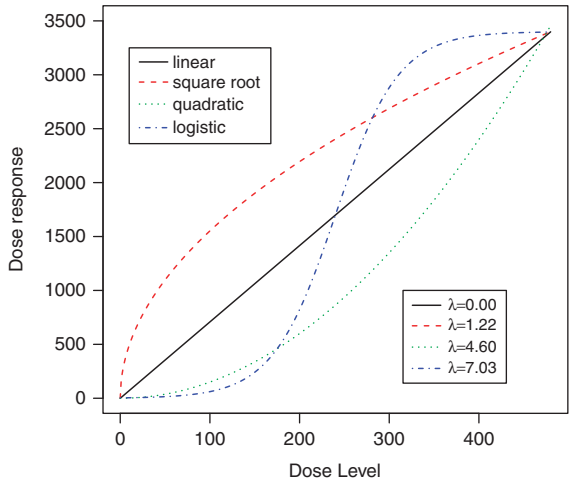

Figure 2. Common patterns of departure from linearity.

4.2. Simulation results

Simulation studies were conducted to evaluate the finite sample performances of the proposed test derived in Section 3. We closely follow the design setting of the example discussed in the previous section, that is, dose levels: $d_1 = 60$ mg, $d_2 = 120$ mg, $d_3 = 240$ mg, and $d_4 = 480$ mg, three dose sequences and three dose periods for each sequence. The configuration of the design is

$$\begin{matrix} d_1 & d_2 & d_4 \\ d_1 & d_3 & d_4 \\ d_1 & d_2 & d_3 \end{matrix} \tag{15}$$

with rows indicating the dose sequences and columns indicating the dose periods. Then, the matrices $\boldsymbol{\Lambda}_1$ and $\boldsymbol{\Lambda}_2$ in (2) are

$$\boldsymbol{\Lambda}_1 = \frac{1}{12}\begin{pmatrix} 4 & 4 & 4 & 4 \\ 4 & 6 & 3 & 3 \\ 4 & 3 & 6 & 3 \\ 4 & 3 & 3 & 6 \end{pmatrix}, \quad \boldsymbol{\Lambda}_2 = \frac{1}{6}\begin{pmatrix} 2 & 0 & 0 & 0 \\ 0 & 3 & 0 & 0 \\ 0 & 0 & 3 & 0 \\ 0 & 0 & 0 & 3 \end{pmatrix}$$

In our simulations, we considered the four dose response patterns specified in (11) through (14). We choose $\lambda_0 = 1.22$, which means that the logistic pattern (14), the quadratic pattern (13), and the square root pattern (12) correspond to the null hypothesis in (4), whereas the linear pattern (11) corresponds to the alternative hypothesis.

We used the square root pattern (12) to illustrate how the data were generated and the simulation process under the other three patterns described above are similar. First, the mean dose response μ_i was determined via Equation (14) as $\boldsymbol{\mu} = (1202.10, 1700.02, 2404.19, 3400.04)'$ and then the $3 \times 3 = 9$ parameters α_{ij} in (1) were set as the corresponding estimated values based on the example data. Precisely,

$$\begin{pmatrix} \alpha_{11} & \alpha_{21} & \alpha_{41} \\ \alpha_{12} & \alpha_{32} & \alpha_{42} \\ \alpha_{13} & \alpha_{23} & \alpha_{33} \end{pmatrix} = \begin{pmatrix} 32.96 & 70.87 & 323.76 \\ -15.56 & -49.35 & -323.76 \\ -17.41 & -70.87 & 49.35 \end{pmatrix}$$

Check that $\sum_{j=1}^{J} \alpha_{ij} = 0$ is indeed satisfied for any i, $i = 1, 2, 3$. Then the resulting mean dose response matrix corresponding to the configuration (15) is given by

$$\begin{pmatrix} 1235.06 & 1770.89 & 3723.80 \\ 1186.54 & 2354.84 & 3076.28 \\ 1184.69 & 1629.15 & 2453.54 \end{pmatrix}$$

Then, we generated y_{ijk} by adding random components $d_i(e_{jk} + \varepsilon_{ijkl})$ to the mean dose response, assuming $\sigma_s = 2.26$, $\sigma_e = 0.83$.

The probability of rejecting H_0 in (4) (i.e. claiming departure from linearity as being minor) under the four dose response patterns were estimated based on 10 000 simulation runs and the results are presented in Table II for $n = 6$, 10, 14, 18.

Table II. Estimated size or power with $\lambda_0 = 1.22$ for testing (4).

	Size			Power
N	Logistic $\lambda = 7.03$	Quadratic $\lambda = 4.60$	Square root $\lambda = 1.22$	Linear $\lambda = 0.00$
6	0.0000	0.0000	0.0529	0.5818
10	0.0000	0.0000	0.0502	0.8619
14	0.0000	0.0000	0.0518	0.9638
18	0.0000	0.0000	0.0470	0.9888

The simulation results indicate that the proposed test has a controlled type one-error rate and a power of at least 85% when there are 10 or more subjects per dose sequence.

5. DISCUSSION

Law [7] briefly described a way to test the departure from dose linearity based on a parametric regression model by focusing on some particular dose response curves. Similar method was also discussed in Senn [16]. The approach we adopt in this paper is essentially an ANOVA model approach, which enables us to detect more patterns of departure from dose linearity. As is true for any ANOVA method, the test we propose is an overall test of departure from dose linearity and it does not directly identify where the departure occurs. The latter question could be addressed by testing whether ϕ_i is close to 0 using a *t*-type test for each *i*, $i = 1,\ldots, I-2$, followed by an adjustment to control for the family wise error rate due to multiple testing. Alternatively, the locations of departure from dose linearity could be identified based on the asymptotic confidence region for $\boldsymbol{\phi}$ provided in Section 3.3.

As a quadratic form of ϕ_is, the parameter λ can be viewed as a squared Mahalanobis distance between $\boldsymbol{\phi}$ and $\mathbf{0}$. Although other choices of λ, for example, $\sum_{i=1}^{I-2} \phi_i^2/(\sigma_s^2+\sigma_e^2)$, are possible, the tests based on such choices are generally complicated and conservative. Geometrically, the distance measure λ defined in this paper can be viewed as the total curvatures of the dose response curves (see Figure 2). Therefore, the proposed test is more powerful in detecting more 'wiggled' departure patterns.

In Section 4.1, we describe how to choose the tolerance λ_0 empirically based on some common dose response patterns when the number of dose levels, the number of dose sequences, and dosing periods have been determined are pre-specified, and σ_s and σ_e can be estimated from pilot data. However, the clinical relevance of such a λ_0 must be justified by clinicians before it can be used in assessing departure from dose linearity. Alternatively, when pilot data are available, an asymptotic confidence interval for λ could be constructed as is performed in Section 3.3, and based on this confidence interval, a reasonable value of λ_0 could be selected for subsequent studies.

As mentioned in Section 2, although dose proportionality is a desirable property, confirming it statistically requires testing H_1: $\lambda = 0$, an alternative consisting of a single value. A test of such a hypothesis will have power equal to the size. On the other hand, from a practical point of view, for prediction purpose, it is often adequate to confirm that the dose response curve is almost linear, or equivalently, the departure from linearity is at most 'minor', which clearly includes dose proportionality as a special case. As shown in this paper, testing for minor departure from dose linearity is meaningful in both statistical and practical sense. Therefore, we recommend testing for minor departure from dose linearity instead of dose proportionality be considered in clinical and PK utility.

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