Application of maximum contrast method to biomedical data using SAS/MULTTEST®

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Abstract

The maximum contrast method was introduced to elucidate dose-response shape and the examples of SAS programs utilizing the MULTTEST® procedure were demonstrated for both quantitative and binomial data, especially, in animal toxicity studies and phase II clinical trials. We also evaluated the statistical power of the maximum contrast method. It was concluded that this method has almost equivalent statistical power to the linear regression method, when the true dose-response curve is monotonic increasing or decreasing. For non-monotonic dose-response relationships, this method has considerably higher power, compared to the regression method.

1.Introduction

One of the typical forms of experiments adopted to biomedical or toxicological studies is to measure a response variable in animals or human beings at several doses of a test chemical or a drug. One purpose of such experiments is to evaluate the dose-response relationship, or, in special cases, to identify a key dose such as the minimum effective dose or the no adverse effect level. Although numerous methods to analyze data in such experiments have been proposed in the articles, conception of contrast plays a particularly important role. Ruberg(1989) pointed out that widely used methods to identify minimum effective dose, such as the Dunnett method and Williams method, are based on a family of a-1 contrasts. Independently of Ruberg, Hirotu noted the max-t method(Hirotu(1979)) , which uses a studentized maximal contrast as the test statistic, was also useful to identify minimum toxic dose. Yoshimura et al. (1997) redefined the maximum contrast method and the above methods are classified as a maximum contrast method.

Although the above-mentioned studies are focused on MED or MTD, similar methodologies are applicable to identify a plausible dose-response pattern in clinical trials and non-clinical trials. In fact, Kishimoto and Hamada(1994) provided an integration program on SAS to choose the most plausible dose-response pattern which best fits data in Phase II clinical trials among a family of candidate patterns, at the same time, giving p-values on the null hypothesis of no-dose patterns. Hamada et al.(1997) also proposed a maximum contrast method for analyzing the dose-response shape in repeated animal toxicity studies. This method expresses several dose-response patterns as a number of contrasts. To overcome the problem of multiplicity using several contrasts simultaneously, the resampling method is applied to calculate an adjusted p-value. For this purpose, SAS/MULTTEST® is a very convenient tool. We introduce the maximum contrast method and show the result of application to actual biomedical data in this presentation, including the coding of SAS/MULTTEST. The performance of the method is also demonstrated using a simulation study.

2.The maximum contrast method

In this article, in order to avoid unnecessary complicated expressions, the mathematical formulas are described, assuming balanced sample cases. SAS/MULTTEST, however, can calculate properly even for any unbalanced cases.

Suppose that an individual animal or human being is randomly allocated to one of the groups with doses($d_1,d_2,...d_a$) of a chemical and a response variable is observed in each individual, indicating a random variable Y_{ij} for the j-th individual(j=1,2,...,n) in the i-th group. For simplicity, assume that Y are independently and normally distributed with $E[Y_{ij}]=\mu_i$ and $V[Y_{ij}]=\sigma^2$ and $Y_{i\cdot}$ being the mean in the i-th group. Suppose further that the first group corresponding to dose d_1 is a control(placebo) group and the sequence $d_1,d_2,...d_a$ is monotone increasing.

Let a contrast statistic Z with a weight $c=(C_1,C_2,...C_a)$, $C_1+C_2+...+C_a=0$ be defined as

$$Z=\sum_i C_i Y_{i\cdot}/\sqrt{\sigma^2\sum_i C_i^2/n}$$

where $s^2=\sum\sum(Y_{ij}-Y_{i\cdot})^2/(N-a)$

When a set of weights $c_k=(C_{k1},C_{k2},...,C_{ka})$;k=1,2,...,m is given, one can obtain corresponding m contrast statistics($Z_1,Z_2,...Z_m$). It is proposed to define the maximum contrast method(MCM) as the one which uses the statistic Z defined as:

$$Z=\max(Z_1,Z_2,...Z_m)$$

to choose a dose-response pattern which best fits observed data among a set of patterns. Examples are given for the case of a=4.

Example 1 Repeated toxicity study

Dose range in toxicity studies is usually much wider than that in clinical trials and more variety of dose-response patterns can be considered. In these studies, we have an interest to look for the initial dose, where adverse response emerges, and the point, where its response saturates. One way to meet this requirement is to choose a dose-response pattern, which best fits observed data from a set of candidate patterns. This idea is realized by preparing the contrast statistics with the following weights corresponding to the candidate patterns:

@@@@efficients content

C @L M H @ @

G H@ LR@LP@@P@R@@

G H@ LT@LP@@R@R@@ L G @ @ @ @ H

G H@@LR@@P@P@@P@@ L G @ @ @ @ H

G H@@LV@LT@LP@PR@ G @ @ H

G H@ LR@@LR@P@@T@@ L G @ @ H

G H@@@LP@LP@LP@R@@ L G @ @ H

For example, contrast (b) can detect the pattern in which response increases linearly from the control to the middle dose and saturates at the @middle dose. A brief comment is added about the contrast (d). In typical toxicity studies, experimental doses of the test compound are selected as a geometrical series, like 0,1,3,10 in the Table 1. The contrast (d) is used, for the purpose of detecting an exponential dose-response pattern, in which the response is directly proportional to the actual dose.

Example 2 Phase II study

In phase II studies, determining the minimal dose which achieves the maximum efficacy, is often required, relating to the selection of the dose for the phase V study. To investigate dose-response relationships and such point, the following set of contrasts are useful:

P L M H @ @

G H@ LR@LP@@P@R@@

G H@ LT@LP@@R@R@@ L G @ @ @ @ H

G H@@LR@@P@P@@P@@ L G @ @ @ @ H

If dose-response shape is m-end type, it is reasonable that the dosage in the phase Vstudy is decided at the middle dose. While in the case of the l-end type of dose-response relationship, we should choose the low-dose in the phase Vstudy. If dose-response shape has linear shape, the high dose should be selected, unless the high dose appears to be unsafe.

3.P-value adjustment for arbitrary contrasts by resampling method

The p-value of Z statistics can be provided by the technique of multiple integration or the exact permutation method. If Z is not significant, two possibilities are considered, 1)intended dose-response relationship does not exist, 2) observed D-R relationship arises from random variation and seems to be meaningless. For this reason, the evaluation of the significance is important. If hypothesis tests for several contrasts are carried out independently, then the probability of declaring a particular contrast significant is ¿ say 0.05, under the null hypothesis, but the probability of declaring at least one of the several tests significant is actually much greater than 0.05. Well-known traditional methods, such us the Bonferroni and the Scheffe method, are known to control the overall significant level for arbitrary contrasts, and be computationally quick. However, they can be overly conservative, so have low power. A new method which adjusts p-value for several contrasts properly using the resampling method is proposed. The theory based on this method is very simple, however the calculation needs high-speed computers and is sometimes time-consuming. SAS/MULTTEST, which was originally produced by Westfall and Young(1992), is a special software for the resampling methods.

Example 1 Repeated toxicity study (quantitative data)

Table 1 demonstrates RBC(Red Blood Cell counts) data from an actual toxicity study. Each group includes 10 rats. It was shown that RBC were reduced by the treatment of the drug. The application of the maximum contrast method is illustrated using this data.

Table 1 @RBC data

f @ @@c	Raw @data(~10^4/mm^3)	Mean	SD
b @@@O	XQT@XPV@XPQ@XPQ@XSX@XOW@XOW@XWX@XRP@XOX	**926.0**	**25.7**
L @@P	WXW@XQT@XOW@WVR@XOW@XSP@WXR@XQO@XQQ@XRP	**911.9**	**20.1**
L @@R	WVS@WVU@XPU@XOW@WVR@WOV@WVS@XPX@XTQ@XPU	**891.5**	**39.8**
L @PO	WUX@XPX@WVS@WTQ@WRO@XOU@XPS@WXW@XRR@XRT	**893.0**	**35.4**

An example of SAS coding is demonstrated in Program 1. In PROC MULTTEST statement, PERMUTATION option specifies calculation of adjusted p-value using the permutation method without replacement, NSAMPLE=option decides the number of resampling and SEED=option determines the initial seed of the random number. P@ROC MULTTEST can provide an adjusted p-value for several contrasts combining TEST statement with several CONTRAST statements.

Program 1　Analysis of toxicity study data

```
     @   Z
   @      \P@  @SZ
   @ \P@  @POZ
 @@@      @ @__Z       Z   Z   Z
      Z
 XQT@XPV@XPQ@XPQ@XSX@XOW@XOW@XWX@XRP@XOX
 WXW@XQT@XOW@WVR@XOW@XSP@WXR@XQO@XQQ@XRP
 WVS@WVU@XPU@XOW@WVR@WOV@WVS@XPX@XTQ@XPU
 WUX@XPX@WVS@WTQ@WRO@XOU@XPS@WXW@XRR@XRT
 Z
     @        @    \    @
        \POOOO@    \SXWXZ
     @    G HZ
      @@      Z
         @F        F@LR@LP@P@RZ
         @F L   F@@LR@@P@P@PZ
         @F L   F@@LT@LP@R@RZ
         @   '  @@@@@LV@LT@LP@PRZ
         @F L      FLR@LR@P@TZ
         @F L      FLP@LP@LP@RZ
    Z
```

OUTPUT 1 shows the output of SAS/MULTTEST. Raw-p stands for non-adjusted p-value and Perm-p is adjusted p-value for multiplicity of six contrasts with 10,000 times of resampling(permutation). The contrast m-end has the smallest Perm_p. We also calculated the model fitness(MF) of each contrast, which indicates the proportion of between sum of squares, explained by the contrast. Since MF of the contrast m-end is 99% and p-value after adjustment(0.0177) is less than 0.05, the dose-response curve can be considered as being saturated at the middle dose.

It is also demonstrated that the adjusted p-value is about 2-3 times larger than the raw one. Since six contrasts have high correlation with each other, the Bonferroni method, which multiplies raw p-value by the number of contrasts, is conservative beyond necessity.

OUTPUT 1　Analysis of toxicity study data　@@@@@@@@@@@@@@@@@@@@@@@@@

```
                     ltkssdrs@s'akdr
 @@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@
        @@@@@@@@@@@@@@@q   ~  @@@@@@@@@o    ~
       @@@@@@@@@@@@@@@@OMOPOS@@@@@@@@OMOQTW
  L   @@@@@@@@@@@@@@@@@OMOQQS@@@@@@@@OMOTRQ
  L   @@@@@@@@@@@@@@@@@OMOOUV@@@@@@@@OMOPVV
    @@@@@@@@@@@@@@@@@@@OMOSQU@@@@@@@@OMPOPW
  L     @@@@@@@@@@@@@@@OMOQPU@@@@@@@@OMOTPT
  L     @@@@@@@@@@@@@@@OMPSXP@@@@@@@@OMRQSX
```

Example 2　Phase II study(binominal data)

Table 2 shows the result of an actual　U study in Japan.

Table 2　Phase　Ustudy data

	Placebo	Low	Middle	High
Improved	20	25	27	29
Not improved	16	16	9	6
Proportion	55.6	61.0	75.0	81.9
Total	36	41	36	35

@@Since the response variable is binomial(improved or not improved), the Cochran-Armitage type statistical test is applicable. The most important goal of this study is to determine the dose in phase　Vstudy. Therefore we are interested in elucidating the saturating point. An example of the program is shown in Program 2.

CA option in the TEST statement specifies performing the Cochran-Armitage test . UPPERTAILED is a keyword to perform one-tailed(increasing response rate with increasing dose) tests. The result is shown in OUTPUT 2.

Program 2 Analysis of Phase　U study data

```
     @     QZ
   @      \P@  @SZ
   @ \O@  @PZ
 @@@      @ @__Z       Z   Z   Z
      Z
 PU@QO@@PU@QT@@X@QV@@U@QX
 Z
     @        @    \      Q@@
        \POOOO@    \TXURZ
     @  G N           HZ    @ Z
      @@      Z
         @F        F@LR@LP@P@RZ
         @F L   F@@LR@@P@P@PZ
         @F L   F@@LT@LP@R@RZ
    Z
```

Output 2 Analysis of Phase　U study data

```
                     ltkssdrs@s'akdr
 @@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@@
 b       @@@@@@@@@@@@@@@q   ~  @@@@@@@@@o    ~
 @      @@@@@@@@@@@@@@@@OMOOQX@@@@@@@@OMOOTU
 @ L   @@@@@@@@@@@@@@@@@OMOROT@@@@@@@@OMOURU
 @ L   @@@@@@@@@@@@@@@@@OMOOST@@@@@@@@OMOPOV
```

@@The most significant contrast is the linear and the adjusted p-value of this contrast is highly significant (p<0.01). If toxicity at the high-dose is negligible, it can be concluded that the high dose should be selected in phase　Vstudy.

4.Evaluation for statistical power of the maximum contrast method

@ 4.1 Method

@ Statistical power of the maximum contrast method(MCM) was investigated, when the combination of the contrasts shown in Example 1 was adopted in 3 dosed toxicity studies, and it was compared with that of the regression method. In this paper, statistical power is defined as the probability to detect any significant dose-response relationships under alternative hypotheses.

1) Number of groups:4
2) Sample size per group:10(equal sample size)
3) Alternative hypotheses: Expected values of each group, ° P C ° Q C ° R@ ° S @shown in Table 3. Random variable, following normal distribution is generated using the RANNOR function. Variance of random variable is fixed at one(known). Alternative hypotheses from 1 to 6 correspond to the elements of dose-response patterns in the MCM. The dose response 'l_m' has a step-type inflation between ° Qand ° 3. No.8 is a quadratic function and No.9 and No.10 are downtown types, these three patterns are non-monotonic dose-response relationships.
4) Number of simulation: 100000 times of each alternative hypothesis
5) Applied methods: The maximum contrast method(MCM), regression method(REG) and Optimal method(OPT) The regression method only uses the contrast (-3,-1,1,3). Optimal method is theoretically the most powerful one under each alternative hypothesis, the coefficients of the optimal contrast shown in Table 3.
6) Significant level: two-tailed 5% Calculation of p-value using resampling method is time-consuming, especially for the purpose of simulation. Therefore critical value of each method is calculated under the assumption of the normal distribution and homogeneity of variance (MCM(2.36) REG(1.96) OPT(1.96)). The Z-value is compared to the critical value to judge statistical significance.

@

4.2 The result of simulation

Table 4 demonstrates the results of simulation. In this table, when ¢equals 0, the figure corresponds to the size of type I error, otherwise it stands for the statistical power. The Type I errors of three methods are controlled at the 0.05 nominal level.

1) The regression method has greater power than the maximum contrast method for three dose-response patterns, linear(1) m-end(4) and m-start(5). The difference is at most 5%. Compared with OPT, the power of MCM is 7% inferior in the worst scenario.
2) MCM and REG has almost the same performance for exp(2), and these figures are usually less than that of OPT by about 5%.
3) For l-end(3) and h-start(6) type dose-response relationships, MCM has greater power than REG, the maximum difference is greater than 10%. The difference between MCM and OPT is not greater than 10%.
4) The regression method has more power than MCM for the step type function l-m(7), because MCM does not include the contrast to detect such a dose-response relationship. The difference is, however, at most 5%, and compared to OPT, the loss of power is 10%
5) When the dose-response curve is quadratic(8), the power of REG is stable at 5%, even increasing Δ. In contrast, however, compared to OPT, the loss of power in MCM is non-ignorable, MCM shows moderately high power.
6) MCM has much greater power than REG for the downturn dose-response patterns, especially in No. 9, the maximum difference is 20%.

@ Summarizing these results, for several monotonic dose-response relationships, the preference of detectable pattern is slightly different between MCM and REG, the difference of power is, however, considerably small. It can be concluded that the power of MCM is almost equivalent to that of the regression method, and compared to the most powerful method, the loss of power is not so high. For non-monotonic dose-response patterns, the power of MCM is higher than the regression method. In other words, MCM has robustness for violation of the assumption of monotonic dose-response.

5.Discussion

In this article, the maximum contrast method was introduced as a method to analyze dose-response pattern statistically for biomedical data. It was confirmed that at least, the power of the MCM is not inferior to that of the regression method and the loss of power, compared to the optimal method is considerably small in the usual situations of toxicological studies. It is not a surprising phenomenon, because of the high correlation among six contrasts. The following fact indicates another evidence of the high correlation. The critical value of the Dunnett test at two-tailed 0.05 in the three dosed experiment is 2.35(DF=) and this value is almost the same as the critical value of the MCM, 2.36. Even though, The Dunnett test utilizes only three contrasts -1 1 0 0, -1 0 1 0 and -1 0 0 1, on the other hand, the MCM consists of the six contrasts.

@

@
@ s @R@' @

m	b @ @cLq	m	° P C ° 0 C ° C ° S	@ @
			¢ GLPKLPNRKPNRKF	LRKLPKPK
			¢ GLPKLOMwKLOMSKF	LVKLTKLPKF
	@ @ L	L	¢ GLPKPKPKF	LRKPKPK
	@ @l L	L	¢ GLPKOKPKF	LTKLPKRK
	L	L	¢ GLPKLPKOKF	LRKLRKPK
	L	L	¢ GLPKLPKLPKF	LPKLPKLPK
	L @ @l L	L	¢ GLPKLPKPKF	LPKLPKPK
			¢ GLPKPKPKLF	LPKPKPKL
			¢ GLPKOKPKC	LPKOKPK
F			¢ GLPKOKPKOMT	L C L C C

¢ YO' P@@ @OMP
s @S@@q @ @

			Δ										
No.	D-R shape	Method	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
1	linear	MCM	0.049	0.072	0.138	0.261	0.420	0.599	0.759	0.877	0.947	0.982	0.994
1	linear	REG	0.050	0.077	0.155	0.293	0.469	0.655	0.806	0.910	0.964	0.989	0.997
1	linear *	OPT	0.050	0.077	0.155	0.293	0.469	0.655	0.806	0.910	0.964	0.989	0.997
2	exp	MCM	0.049	0.072	0.142	0.269	0.440	0.628	0.788	0.901	0.961	0.988	0.997
2	exp	REG	0.050	0.075	0.147	0.274	0.440	0.619	0.773	0.886	0.950	0.983	0.995
2	exp	OPT	0.051	0.079	0.165	0.317	0.505	0.696	0.840	0.933	0.977	0.994	0.999
3	l-end	MCM	0.049	0.073	0.149	0.293	0.484	0.689	0.846	0.941	0.982	0.996	0.999
3	l-end	REG	0.050	0.072	0.135	0.247	0.395	0.565	0.719	0.844	0.924	0.970	0.988
3	l-end	OPT	0.049	0.084	0.195	0.376	0.591	0.780	0.907	0.971	0.992	0.999	1.000
4	m-end	MCM	0.049	0.075	0.152	0.295	0.478	0.674	0.830	0.929	0.976	0.994	0.999
4	m-end	REG	0.050	0.080	0.166	0.318	0.506	0.697	0.842	0.933	0.977	0.994	0.999
4	m-end	OPT	0.050	0.083	0.181	0.349	0.551	0.746	0.880	0.957	0.987	0.997	1.000
5	m-start	MCM	0.049	0.075	0.153	0.295	0.480	0.676	0.831	0.929	0.976	0.994	0.999
5	m-start	REG	0.050	0.080	0.166	0.318	0.506	0.697	0.842	0.933	0.977	0.994	0.999
5	m-start	OPT	0.051	0.083	0.181	0.350	0.553	0.747	0.881	0.955	0.987	0.997	0.999
6	h-start	MCM	0.049	0.074	0.149	0.294	0.489	0.691	0.846	0.941	0.982	0.996	0.999
6	h-start	REG	0.050	0.072	0.135	0.247	0.395	0.565	0.719	0.844	0.924	0.970	0.988
6	h-start	OPT	0.050	0.086	0.195	0.375	0.591	0.783	0.906	0.969	0.992	0.998	1.000
7	l-m	MCM	0.049	0.081	0.178	0.358	0.579	0.785	0.913	0.974	0.994	0.999	1.000
7	l-m	REG	0.050	0.089	0.203	0.396	0.617	0.808	0.923	0.977	0.995	0.999	1.000
7	l-m	OPT	0.050	0.098	0.241	0.475	0.714	0.885	0.966	0.993	0.999	1.000	1.000
8	quadratic	MCM	0.049	0.065	0.117	0.214	0.361	0.548	0.732	0.877	0.955	0.988	0.998
8	quadratic	REG	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.051	0.049	0.050
8	quadratic	OPT	0.050	0.096	0.245	0.474	0.714	0.887	0.966	0.994	0.999	1.000	1.000
9	downturn1	MCM	0.049	0.061	0.096	0.165	0.266	0.397	0.545	0.690	0.811	0.898	0.949
9	downturn1	REG	0.050	0.059	0.087	0.135	0.204	0.292	0.395	0.507	0.620	0.722	0.806
9	downturn1	OPT	0.049	0.073	0.145	0.269	0.428	0.609	0.765	0.880	0.948	0.980	0.994
10	downturn2	MCM	0.049	0.067	0.119	0.217	0.357	0.524	0.691	0.826	0.917	0.967	0.989
10	downturn2	REG	0.050	0.068	0.121	0.215	0.343	0.493	0.644	0.775	0.874	0.940	0.973
10	downturn2	OPT	0.049	0.076	0.153	0.288	0.460	0.647	0.799	0.906	0.963	0.988	0.997

@ MCM: Maximum contrast method REG: regression analysis(-3,-1,1,3) OPT:optimal method
@ *:The result is same as that of REG. Because the optimal contrast is -3,-1,1,3 in this case.

Other statistical methods are known to detect dose-dependency e.g. MAX-t(Kuriki Hirotu and Hayter(1989)) and the Williams test(Williams(1971)). Kuriki Hirotu and Hayter(1989) showed that these methods also have equivalent power to the regression method. The relation between the proposed method and these methods is discussed. Yoshimura et al.(1997) expressed the MAX-t method using the contrasts. The test statistics of this method is the maximum value of the following three contrasts for three dosed experiment.

-3 1 1 1, -1-1 1 1 and -1 -1 -1 3

Yoshimura et al.(1997) also modified the maximum contrast statistics shown in formula (1), extending the definition of denominator and suggested that the Williams method can also be expressed using the several contrasts. The coefficients of the contrasts in comparing the highest dose group with the control are as follows.

-1 0 0 1, -2 0 1 1 and -3 1 1 1

It should be noted that the proposed MCM includes the contrast -3 1 1 1 and -1 -1 -1 3 in the MAX-t method, and -3 1 1 1 in the Williams method. As predicted from this point, and the fact that the loss of power is small, comparing with the most powerful method, although the preference of detectable dose-response patterns are different among these methods, the difference of statistical power is not so high. The MAX-t method is produced to detect step-type changes in dose-response relationships and the purpose of the Williams method is to detect the minimum significant dose, assuming monotonic response. These two methods do not show direct information on the dose-response shapes. In contrast, since the proposed method makes a model of dose-response pattern, explicitly using a contrast, the MCM has the advantage of interpretability for judging the dose-response pattern.

Although we showed the example of the set of contrasts in phase II clinical trials and toxicological studies, it is not intended to propose a definitive solution. The choice of a set of contrasts should be flexible, depending on biologically plausibility. SAS/MULTTEST can calculate adjusted p-value for the arbitrary set of contrasts using the resampling method.

6. Conclusion

The usefulness of the maximum contrast method was confirmed, as a statistical method to decide the dose-response pattern. We are now applying this method to accumulated repeated toxicity studies and phase II clinical trials data to evaluate the consistency with the judgments of experienced experimenters on the dose-response shape. In general, there is a considerably high consistency between the results of MCM and the judgments of investigators.

Several practical problems remain unsolved, for example, in the cases of unexpected D-R relationships (such as a downturn type), treatment of outliers(especially in toxicity studies) and sample size design(in clinical trials). We continue to investigate application of the maximum contrast methods to actual biomedical study data.

Trademarks

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