HOW TO MEASURE PAIN IN ANIMALS

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Master Thesis in Statistics, Spring 2010.

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Abstract

This paper provides analyses on how to measure pain in animals. It contains two parts, how to measure the pain and how to analyze data.

Since animals cannot tell the amount of pain they are feeling, indirect measurement is needed. The experiment studied in this paper concerns guinea pigs. The animals have pain in their shoulders. The amount of pain is estimated by measuring the pressure under the pawns when they stand on a slanting board.

First we study different estimators of pain. We use Taylor expansion to prove theoretical result, and do simulation based on R to support the theoretical work. An ambition is to find an estimator that is unbiased and lower variance. Then, we study different statistical tests and choose the one with largest power. At last we do a simulation to demonstrate the results.

We can do research in the future by using the method in this paper, but not use real data since this is only a research to find a method with the simulation. We will find the best statistic and most efficient statistical test.

Contents

1. Introduction:

People and animals may get pain, sometimes it is a serious problem. There are several methods to ease pain; one of them is to take treatment with drug. As the development of the science, many new drugs have been produced. However, it's not always easy to know whether these new drugs are useful or not.

Firstly, the new drugs should be safe, which means it won't kill animals and won't sharpen the pain. If the new drug is not safe, we should stop the experiment immediately. The drug is efficient means it can ease the pain quickly and efficiently.

Usually, the new drugs are tested on animals first. If it is proved that there is no harm to animals, we can carry out a new experiment on the small number of people, which is used to test whether these new drugs are safe for people. After that the efficiency test can be done on a group of people. At last, a test on large number of people will show whether this new drug is efficient or not. So it is necessary to measure the pain before take treatment and measure it again after the treatment, some conclusion can be drawn by comparing the results.

However, it is difficult to measure the people's pain since they cannot tell how much pain they feel exactly. For example, a patient feels pain in his arm but he can still lift up a heavy box, while it is difficult to find the point just when he cannot lift up the box. He even cannot describe how he feels about the pain, only can tell if he feels better or worse. It is even more difficult to measure the animals' pain since they cannot speak. All these factors should be considered when designing the research.

We know that when animals get pain in shoulders they cannot push or pull in his full strength. So we can take an indirect way to measure the pain in arms: let them stand on a slanting board and measure the pressure under the pawns. The less pressure we measured, the more pain they have.

From the previous research, assume only one shoulder get pain. We measures the pain between the left and the right shoulder by comparing the pressure difference of two pawns, or calculating the ratio pressure of left and right pawns, measure this ratio both before and after the treatment.

It is not the only way to test the pressure of arms after treatment. There are some other ways, such as comparing the measurements before and after treatment, or comparing the measurement after treatment with the baseline. Linear regression model is also a choice.

The aim to do this research is to find the best way to measure and analyze pain in animals. We will focus on the situation when animals have pain in one shoulder. In chapter 2 we will describe a typical experiment conducted to measure pain. In chapter 3, we will focus on how to combine several measurements into one measurement of pain. In chapter 4, we will study how to measure changes in pain. Finally we summarize our conclusion in chapter 5.

2. A typical experiment:

The experiment concerns how to measure pain in the shoulders on animals (guinea pigs). By injecting a substance (monosodium iodoacetate) in their left shoulders, the animals will suffer from pain in these shoulders. To measure the degree of pain, the animal is placed on a slanting board. The board can register the pressure under each pawn.

·The definition of the variables:

L = pressure measured under the left pawn (g)

R = pressure measured under the right pawn (g)

Suppose we have 100 guinea pigs, and in order to get more reliable result, measurement for each guinea pig repeats six times. The data structure will be as shown in Table 1.

Table 1: Measurement at the beginning of the research: These measurements come from one guinea pig at the beginning of the research. L1 is the pressure of the left pawn when we measure it first time, R2 is the pressure of the right pawn when we measure it second time. L is the average of L1, L2, L3, L4, L5, and L6. Ratio 3 equals L3/R3.

	Measure1	Measure2	Measure3	Measure4	Measure5	Measure6	Average
Left	L1	L2	L3	L4	L5	L6	L
Right	R1	R2	R3	R4	R5	R6	R
Ratio	Ratio 1	Ratio 2	Ratio 3	Ratio 4	Ratio 5	Ratio 6	

A statistical question is how to combine this data into a summary measurement of the animal's pain.

·The ratio is calculated from this table by dividing the L by R. Define it as:

$$K = \frac{L}{R} \qquad (1)$$

This table only shows the measurements at the start of the research. We can calculate K from this table at the beginning of the research.

3. An estimator of pain

An obvious way to estimate pain is to calculate the ratio of the pressure under each pawn. There are two ways to calculate the ratio. One is to calculate the ratio of the sum for each pawn (M_A), the other way is to calculate the mean of all ratios (M_B).

$$M_A = \frac{\sum L_i}{\sum R_i} \quad M_B = \frac{1}{n}\sum \frac{L_i}{R_i} \qquad (2)$$

Two important characteristics of an estimator are that it should be unbiased and has a lower variance.

3.1 Unbiased

Use a Taylor series expansion of x / y around u_x and u_y

$$\frac{x}{y} \approx \frac{x}{y}\Big|_{u_x,u_y} + (x-u_x)\frac{\partial}{\partial x}(\frac{x}{y})\Big|_{u_x,u_y} + (y-u_y)\frac{\partial}{\partial y}(\frac{x}{y})\Big|_{u_x,u_y} + \frac{1}{2}(x-u_x)^2\frac{\partial^2}{\partial x^2}(\frac{x}{y})\Big|_{u_x,u_y} \quad (3)$$
$$+\frac{1}{2}(y-u_y)^2\frac{\partial^2}{\partial y^2}(\frac{x}{y})\Big|_{u_x,u_y} + (x-u_x)(y-u_y)\frac{\partial^2}{\partial x\partial y}(\frac{x}{y})\Big|_{u_x,u_y} + O(((x-u_x)\frac{\partial}{\partial x} + (y-u_y)\frac{\partial}{\partial y})^3(\frac{x}{y}))$$

A Taylor series expansion of $\bar{x} / \bar{y}$ around u_x and u_y is similar to the equation above. (G.M.P.van K., 1999, p.300-305)

The mean of M_A and M_B can be found by applying the expectation operator to the individual terms[1].

$$E(M_A) = E(\bar{x} / \bar{y}) = \frac{u_x}{u_y} + \mathrm{var}(\bar{y})\frac{u_x}{u_y^3} - \frac{\mathrm{cov}(\bar{x},\bar{y})}{u_y^2} \approx \frac{u_x}{u_y} + \frac{1}{n}(\mathrm{var}(y)\frac{u_x}{u_y^3} - \frac{\mathrm{cov}(x,y)}{u_y^2}) \quad (4)$$

$$E(M_B) = E\{(\frac{\bar{x}}{y})\} = E(\frac{x}{y}) \approx \frac{u_x}{u_y} + \mathrm{var}(y)\frac{u_x}{u_y^3} - \frac{\mathrm{cov}(x,y)}{u_y^2} \quad (5)$$

It is clear from these two expectations that M_A is asymptotically unbiased when $\lim n \to \infty\ E(M_A) = \frac{u_x}{u_y}$. If $\mathrm{var}(y)\frac{u_x}{u_y^3} - \frac{\mathrm{cov}(x,y)}{u_y^2} = 0$, M_B is unbiased estimator of $\frac{u_x}{u_y}$, but in most cases, it does not equals 0, so M_B is not unbiased at all time.

3.2 Variance

An approximation of the variance of M_A and M_B is obtained by using the first order terms of the Taylor series expansion[2].

$$\mathrm{var}(M_A) = \mathrm{var}(\frac{\bar{x}}{\bar{y}}) = E\{(\frac{\bar{x}}{\bar{y}} - E(\frac{\bar{x}}{\bar{y}}))^2\} \approx E\{(\frac{\bar{x}}{\bar{y}} - \frac{u_x}{u_y})^2\} \approx \frac{\mathrm{var}(\bar{x})}{u_y} + \frac{u_x^2\,\mathrm{var}(\bar{y})}{u_y^4} - \frac{2u_x\,\mathrm{cov}(\bar{x},\bar{y})}{u_y^3}$$

$$\approx \frac{1}{n}(\frac{\mathrm{var}(x)}{u_y} + \frac{u_x^2\,\mathrm{var}(y)}{u_y^4} - \frac{2u_x\,\mathrm{cov}(x,y)}{u_y^3}) \quad (6)$$

$$\mathrm{var}(M_B) = \mathrm{var}(\frac{\bar{x}}{y}) = E\{(\frac{\bar{x}}{y} - E(\frac{\bar{x}}{y}))^2\} \approx E\{(\frac{\bar{x}}{y} - \frac{u_x}{u_y})^2\} \approx \frac{1}{n}(\frac{\mathrm{var}(x)}{u_y} + \frac{u_x^2\,\mathrm{var}(y)}{u_y^4} - \frac{2u_x\,\mathrm{cov}(x,y)}{u_y^3}) \quad (7)$$

Estimator M_A and M_B have, only in first order Taylor series expansion, an equal variance.

[1] (4) and (5) is the result from (G.M.P.van K., 1999, p.301)
[2] (6) and (7) is the result from (G.M.P.van K., 1999, p.301)

3.3 Simulation:

Now we will study the characteristics of the estimators by doing a simulation study.

3.31Unbiased

Đo a simulation study as follow to test which one is unbiased.

1) Assume that the sum pressure of Left and right pawn T, which follows the normal distribution. $T \sim N(200,10)$, generate T_1, T_2, T_3 … T_n n is the number of measurements we get each time.
2) Assume that the proportion of the total weight put on the left pawn follows uniform distribution, $(0.25, 0.4)$, generate L_1, L_2, L_3 … L_n. These values stand for the pressure in the left pawn.
3) The weight on the right pawn is the total weight minus that on the left pawn.
4) Calculate the mean of M_A and M_B.
5) Repeat the loop 1000 times.
6) Calculate the true value of the mean value.

$$\frac{200\times[(0.25+0.4)/2]}{200\times[1-(0.25+0.4)/2]}=0.4815$$

7) Store the results,
n is the number of measurements each time, n is from 1 to 1000.

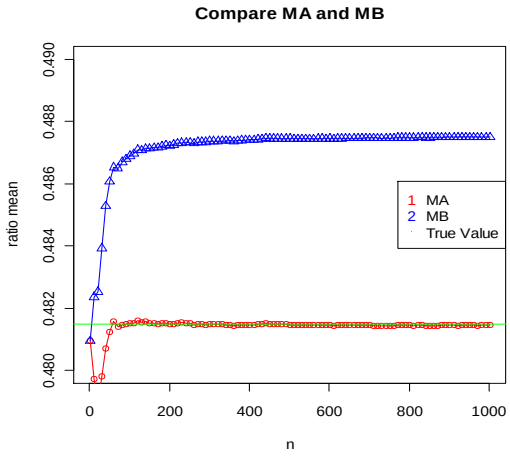

Figure 1: The mean of the ratio: The triangular line is the mean of M_B and the circular line is the mean of M_A, n is the number of measurements we get each time. True value is calculated by the original assumption. When n is small, both M_A and M_B are biased. However, when n approach infinite, M_A approach the true value while M_B does not.

This figure clearly shows that M_A is asymptotically unbiased and M_B is biased. We have mention that the size of the bias depend on whether $\mathrm{var}(y)\frac{u_x}{u_y^3}-\frac{\mathrm{cov}(x,y)}{u_y^2}$ equals to zero. Clearly it doesn't equal to zero this time since M_B is biased.

3.32 Variance :

With the same simulation approach we can now study the variances of the two estimators

Does a similar simulation study as above; everything is same except the step (4), calculate variances of M_A and M_B this time.

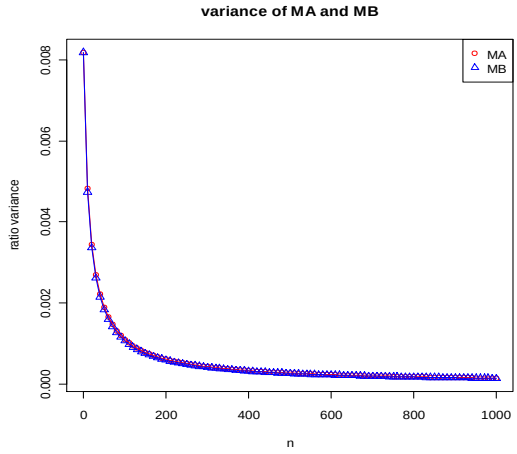

Figure 2: The variance of the ratio: The triangular line is the variance of M_B and the circular line is the variance of M_A, n is the number of measurements we get each time. As n getting larger, variance of M_A and M_B both decrease, these points almost drop in a same line. It is difficult to distinguish them.

It is hard to tell which one is better from this figure. However, from the simulation result, Variance of M_A is always less than variance of M_B when $T \sim N(200,10)$.

If we change the initial assumption, for example, $T' \sim N(200,20)$, The result will change when the assumption change.

Change the assumption again, $T'' \sim N(200,30)$, We get opposite result. So the variances are easily affected by the assumption.

All the results are listed in Table 2

Table 2: Variance of M_A and variance of M_B when variance of T changes. T is the total pressure on the pawns; it is easy to generate large or small values by changing the variance of T. Notice that variance of M_A is not always smaller or larger than the variance of M_B, the order depends on the variance of T. We may even get opposite result by using different T.

T~N(200,10)		T'~N(200,20)		T''~N(200,30)	
var(M_A)<var(M_B)	var(M_A)>var(M_B)	var(M_A)<var(M_B)	var(M_A)>var(M_B)	var(M_A)<var(M_B)	var(M_A)>var(M_B)
100	0	84	16	0	100

Focus on the form of M_A and M_B in the equation (3), we can find that:

$$M_A = \frac{\sum L_i}{\sum R_i} \text{ is easily affected.}$$

$$M_B = \frac{1}{n}\sum \frac{L_i}{R_i} \text{ is more stable.}$$

T' is more fluctuate than T by changing the variance in normal distribution, the variance of T'' is larger than T', so it is more fluctuate. When we do this simulation, the distribution of the proportion of the total weight put on the left pawn does not change, so when we generate the pressure on the left pawn when $T'' \sim N(200,30)$, it is easy to get very large value. All the ratios won't change since the uniform distribution doesn't change, so variance of M_B won't change. However, when we calculate M_A, the numerator and denominator are both affected by the changing of variance of T.

A simple conclusion is

$Var(M_A) < Var(M_B)$ When variance of T is small

$Var(M_A) > Var(M_B)$ When variance of T is large

3.4 Choose the best estimator:

M_A is an unbiased estimator of $\frac{u_x}{u_y}$ and $Var(M_A)$ is smaller when variance of T is small.

M_B is a biased estimator and $\frac{u_x}{u_y}$ and $Var(M_B)$ is smaller when variance of T is large.

The variance of M_A and M_B is nearly the same in the first order Taylor series expansion. So choose M_A in most condition because it is unbiased. However, if the ratio is highly fluctuating, we can choose M_B instead for the lower variance in that condition.

3.5 An alternative estimator of L/R

In this experiment, we use the ratio L/R as a statistic. However, there are other ways to estimate the pain based on these data, we can construct statistic, such as R/L, or $R-L$.

According to the characteristics of estimators which have been mentioned before, we should prove whether R/L and $R-L$ are unbiased and compare the variance.

3.5.1 R/L

It seems no difference between L/R and R/L. However, according to the hypothesis, L stands for the pressure in the left pawn, while R stands for pressure in the right pawn. L is less than R since the left shoulder get pain by MIA injection. So L/R is less than 1, and R/L is larger than 1. L/R is a number between 0 and 1.

The shortage of R/L is that when the left shoulder get so large pains that L approach 0, R/L becomes a large number.

When we test whether R/L is unbiased, it is convenient to form statistic as we have done before. Define that:

$$M_C = \frac{\sum R_i}{\sum L_i} \quad M_D = \frac{1}{n}\sum \frac{R_i}{L_i} \tag{8}$$

It is clear from these two expectations that M_C is asymptotically unbiased when $\lim n \to \infty\ E(M_C) = \frac{u_y}{u_x}$. If $\text{var}(x)\frac{u_y}{u_x^3} - \frac{\text{cov}(x,y)}{u_x^2} = 0$, M_D is unbiased estimator of $\frac{u_y}{u_x}$, but in most cases, it does not equals 0, so M_D is not unbiased at all time.
Do a simulation as above, we can draw a figure:

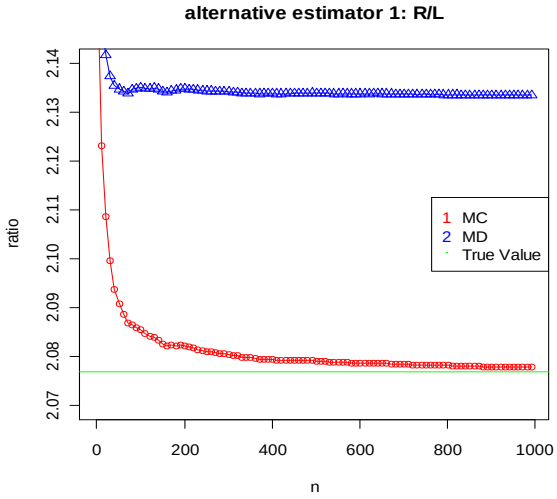

Figure 3: The mean of the R/L. The triangular line is the mean of M_D and the circular line is the mean of M_C, n is the number of measurements we get each time. True value is calculated by the original assumption. When n is small, both M_A and M_B are biased. However, when n approach infinite, M_C approach the true value while M_D does not.

We can make a conclusion that M_c is asymptotically unbiased when $\lim n \to \infty$, while M_d is not unbiased. This conclusion is similar to the situation when we analyze L/R.

To find which one is better, we should compare the variance. However, L/R and R/L are not estimates of the same parameter. So we cannot compare them directly. In this situation we should use Coefficient of variation, which is defined as standard deviation divided by the mean $\frac{\sqrt{\text{var}\,inace}}{mean}$.

Đo a simulation:

Table 3: Compare coefficient of variation of L/R and coefficient of variation of R/L when assumptions change. Compare coefficient of variation of L/R and R/L when the assumption change. The first frame shows do the simulation 100 times, coefficient of variation of L/R is smaller than that of R/L. This result is same in all the condition, which means $coe(L/R) < coe(R/L)$ all the time.

T~N(200,10)		T~N(200,20)		T~N(200,30)	
c(L/R)<c(R/L)	c(L/R)>c(R/L)	c(L/R)<c(R/L)	c(L/R)>c(R/L)	c(L/R)<c(R/L)	c(L/R)>c(R/L)
100	0	100	0	100	0
(0.25,0.4)		(0.3,0.6)		(0.1,0.8)	
c(L/R)<c(R/L)	c(L/R)>c(R/L)	c(L/R)<c(R/L)	c(L/R)>c(R/L)	c(L/R)<c(R/L)	c(L/R)>c(R/L)
100	0	100	0	100	0

It is clearly that coefficient of variation of L/R is always less than that of R/L, even we change the assumptions.

3.5.2 $R-L$

The other way to calculate the relationship between the pains in left and right shoulder is by calculates $R-L$. Assume that $R > L$.

Test whether $R-L$ is unbiased at first. Draw this figure by doing a simulation:

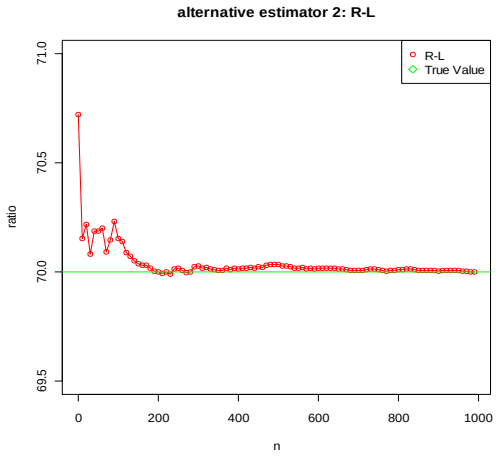

Figure 4: The mean of $R-L$. The circular line is the mean of $R-L$, n is the number of measurements we get each time. True value is calculated by the original assumption. When n is small, $R-L$ are biased. However, when n approach infinite, $R-L$ approach the true value.

So $R-L$ is also an unbiased estimator since the mean of $R-L$ approaches the true value when $\lim n \to \infty$.

L/R and $R-L$ are not estimates of the same parameter. So we cannot compare them directly, use Coefficient of variation.

We do the simulation again. The code is nearly same; only add the part to compare the difference of L and R.

The result is showed in table 4:

Table 4: Compare coefficient of variation of L/R and coefficient of variation of $R-L$ when assumptions change. Compare coefficient of variation of L/R and $R-L$ when the assumption change. The first frame shows do the simulation 100 times, coefficient of variation of L/R is smaller than that of $R-L$. This result is same in all the condition, which means $coe(L/R) < coe(R/L)$ all the time.

T~N(200,10)		T~N(200,20)		T~N(200,30)	
c(L/R)<c(R-L)	c(L/R)>c(R-L)	c(L/R)<c(R-L)	c(L/R)>c(R-L)	c(L/R)<c(R-L)	c(L/R)>c(R-L)
100	0	100	0	100	0
(0.25,0.4)		(0.3,0.6)		(0.1,0.8)	
c(L/R)<c(R-L)	c(L/R)>c(R-L)	c(L/R)<c(R-L)	c(L/R)>c(R-L)	c(L/R)<c(R-L)	c(L/R)>c(R-L)
100	0	100	0	100	0

The coefficient of variation of $R-L$ is larger than ratio statistic L/R even we change the assumptions. It is because that the ratio is less than 1, so the variance and expectation are all small.

Now we have three unbiased statistics L/R, R/L and $R-L$. We would choose L/R as the statistic because it has the lowest coefficient of variation.

4. How to measure changes in pain

4.1 How to calculate ratios.

So far we have studied the situation where pain is measured at one time point. Now we will study how to measure change in pain when the measurements are done at different time points.

Measure the pressure on the pawns at the other time points. We can get the measurements.

The measurements can be illustrating by drawing a figure.

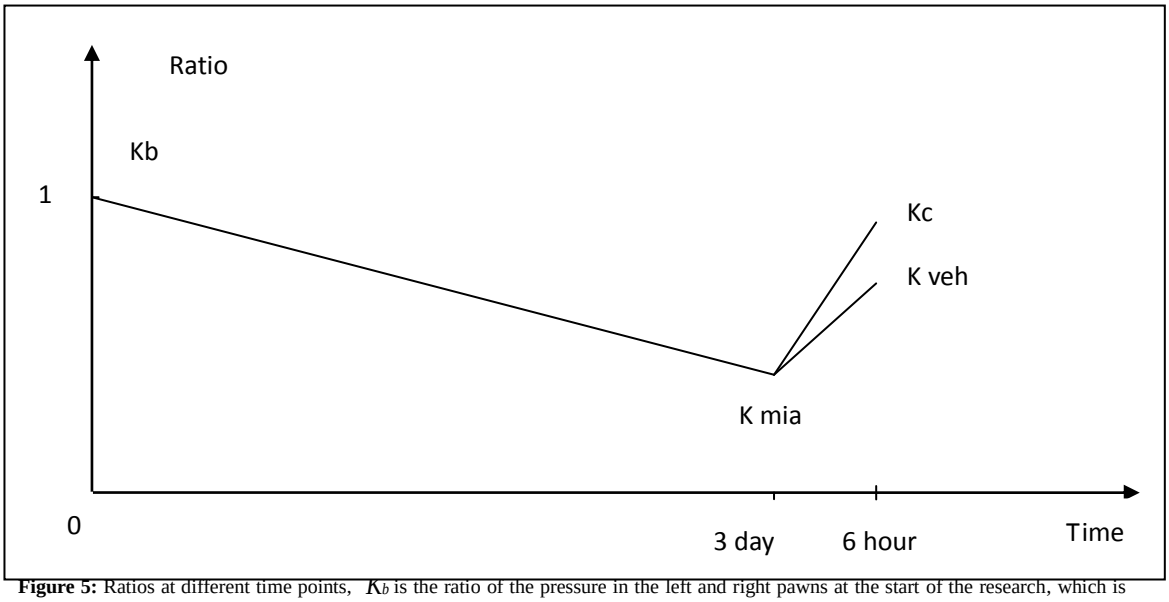

Figure 5: Ratios at different time points, K_b is the ratio of the pressure in the left and right pawns at the start of the research, which is called the baseline in the research. Three days after the MIA injection, the ratio drops to some value, which is K_{mia} , it's time to take compound treatment on one group of the guinea pigs. So K_{mia} is the ratio of the pressure in the left and right pawns just when they are taking the compound treatment. Six hours after compound treatment, this ratio will increase; measure the value of the ratio six hours after the compound treatment, names it K_c . From the experience, the pain will ease as the time goes by without any treatment. So for the group of guinea pigs which have not taken this compound treatment, the ratio of pressure in the left and right pawns will increase too; measure this ratio six hours later too, we call the ratio of those didn't take injection K_{veh} , which stands for vehicle.

K_c and K_{veh} may be higher or lower than initial ratio K_b . If these ratios are lower than 1, it shows that there are still pain in the left shoulder. However, if the ratio is higher than 1, it does not mean that the condition of shoulders get better. It means the pressure on the right pawn is less than that of left pawn. Perhaps it is caused by pushing so hard on the right because of the pain in left shoulder. Then the right shoulder gets pain. Anyway, there are pains in the shoulders.

From these three tables, it is easy to calculate K_c K_{mia} and K_{veh}

Table 4: Measurement three days after MIA injection: These measurements come from one guinea pig three days after MIA injection. L1m is the pressure of the left pawn when we measure it first time, R2m is the pressure of the right pawn when we measure it second time. Lm is the average of L1m, L2m, L3m, L4m, L5m, and L6m. Ratio 3m equals L3m/R3m.

	Measure1	Measure2	Measure3	Measure4	Measure5	Measure6	Average
Left	L1m	L2m	L3m	L4m	L5m	L6m	Lm
Right	R1m	R2m	R3m	R4m	R5m	R6m	Rm
Ratio	Ratio 1m	Ratio 2m	Ratio 3m	Ratio 4m	Ratio 5m	Ratio 6m	

Measure the pressure six hours after compound treatment, we can get other tables.

6 hours after treatment.

Table 5: Measurement 6 hours after treatment: These measurements come from one guinea pig six hours after treatment. L1c is the pressure of the left pawn when we measure it first time, R2c is the pressure of the right pawn when we measure it second time. Lc is the average of L1c, L2c, L3c, L4c, L5c, and L6c. Ratio 3c equals L3c/R3c.

	Measure1	Measure2	Measure3	Measure4	Measure5	Measure6	Average
Left	L1c	L1c	L3c	L4c	L5c	L6c	Lc
Right	R1c	R2c	R3c	R4c	R5c	R6c	Rc
Ratio	Ratio 1c	Ratio 2c	Ratio 3c	Ratio 4c	Ratio 5c	Ratio 6c	

6 hours after treatment for the group which get the vehicle instead of take treatment.

Table 6: Measurement 6 hours later but without treatment: These measurements come from one guinea in the control group which didn't take treatment. L1v is the pressure of the left pawn when we measure it first time, R2v is the pressure of the right pawn when we measure it second time. Lv is the average of L1v, L2v, L3v, L4v, L5v, and L6v. Ratio 3v equals L3v/R3v.

	Measure1	Measure2	Measure3	Measure4	Measure5	Measure6	Average
Left	L1v	L2v	L3v	L4v	L5v	L6v	Lv
Right	R1v	R2v	R3v	R4v	R5v	R6v	Rv
Ratio	Ratio 1v	Ratio 2v	Ratio 3v	Ratio 4v	Ratio 5v	Ratio 6v	

4.2 Significance test for the new drug.

Use all the ratios calculated before, we can test whether the drug is significant effective.

In the reference (Daigen Xu et al., 2008) the authors use the following definition to calculate the percentage of incapacity is calculated by use the formula.

$$(\Delta K_c - \Delta K_{mia}) \Big/ (\Delta K_{veh} - \Delta K_{mia}) \qquad (9)$$

ΔK_c is the difference between the K_c and K_b

ΔK_{mia} is the difference between the K_{mia} and K_b

ΔK_{veh} is the difference between the K_{veh} and K_b

While $\Delta K_c - \Delta K_{mia} = (K_c - K_b) - (K_{mia} - K_b) = K_c - K_{mia}$

And $\Delta K_{veh} - \Delta K_{mia} = (K_{veh} - K_b) - (K_{mia} - K_b) = K_{veh} - K_{mia}$

From the calculation, they don't use the baseline, which is K_b. They just compare K_c and K_{veh} with K_{mia}. We can do some transformation:

$$(K_c - K_{mia}) \Big/ (K_{veh} - K_{mia}) \qquad (10)$$

While K_c K_{mia} and K_{veh} can be measured from the experiment.

If the new drug is efficient, after six hours, the ratio taken treatment should be significant different to the ratio taken no treatment but only wait for six hours.

4.3 Alternative ways to measure the change.

Now we introduce the specific design with K_b K_c K_{mia} K_{veh}, to show that which could be done better. Assume that we want to compare two groups, group one take treatment three days after MIA injection, while group two get the vehicle instead of take treatment. Calculate which of the following approaches is best.

a) Compare K_c and K_{veh} after treatment.

b) Change from K_b : $K_c - K_b$ versus $K_{veh} - K_b$

c) Change from K_{mia} : $K_c - K_{mia}$ versus $K_{veh} - K_{mia}$

d) Use the linear regression model $K_c = a + b_1 K_{MIA} + b_2 Treatment$

Part (a) is what we have done; now we need to simulate data to calculate other three.

For each animal:

1) Generate baseline value K_b.

2) Generate K_{mia}. (It may independent of K_b or not)

3) Generate K_c or K_{veh} which depends on both treatment and K_{mia}.

The linear regression model is defined as $K_c = a + b_1 K_{mia} + b_2 Treatment$. K_c and K_{mia} are generated above. The treatment is a sequence, which equals 1 when the guinea pigs have been taken the treatment, while equals 0 when the guinea pigs haven't been taken the treatment.

Compare the groups based on the four approaches above and see which has the highest power. As we know, the power of the test is one minus probability of a Type II error when the alternative hypothesis is true.

$$power = P(reject H_0 \mid H_1 True) \qquad (11)$$

If we have larger power, it means we get lower Type II error.

4.4 Simulation:

4.41 Weak correlation:

It is the correlation between K_b and K_{mia}

Generate the data for two groups; one will be taken treatment while the other will not.

a) For the group one who will be taken treatment

1) Assume that at the beginning of the research, the sum pressure of Left and right pawn T, which follows the normal distribution. $T \sim N(200,10)$. The proportion of the total weight put on the left pawn follows uniform distribution $(0.45, 0.55)$, which means the ratio is around 1.

2) Assume there is correlation between K_{mia} and K_b, K_{mia} equals to K_b multiplied by H, which follows the uniform distribution $(0.1, 0.7)$.

3) Assume there is correlation between K_c and K_{mia}, K_c equals to K_{mia} multiplied by J, which follows the uniform distribution $(0.95, 1.6)$.

4) Generate the effect factor t, which is a sequence made by 0 and 1. 0 stands for no treatment and 1 stands for treatment.
5) Repeat it 1000 times. Get the sequence of K_b, K_c and K_{mia}. Store the result.
6) Compare the two groups according to the four different methods.
7) Store if significant or not.
8) Compare the power by each method.

b) For the group one who will be not taken treatment

Everything is same except the third step, K_c equals to K_{mia} multiply by P, which follows the uniform distribution $(0.9,1.3)$. The difference here show the effect of the treatment, the group who take the treatment will get better sooner.

The result of simulation:

Table 7: Power of different tests in weak correlation condition, a stands for compare K_c and K_{veh} after treatment, while b means Change from K_b : $K_c - K_b$ versus $K_{veh} - K_b$. C is Change from K_{mia} : $K_c - K_{mia}$ versus $K_{veh} - K_{mia}$ and d is the linear regression model $K_c = a + b_1 K_{MIA} + b_2 Treatment$. Pick up some of the powers to show power of a is always the smallest one, while the power of d is the largest one.

Power	**Different test**	**a**	**b**	**c**	**d**
animals' number					
6		0	0.01	0.26	0.27
10		0	0	0.46	0.54
20		0.02	0.02	0.87	0.95
30		0.05	0.02	0.98	0.99
40		0.09	0.09	0.99	1
50		0.11	0.19	1	1
60		0.29	0.36	1	1
70		0.41	0.34	1	1
80		0.47	0.56	1	1
90		0.56	0.56	1	1
100		0.63	0.65	1	1

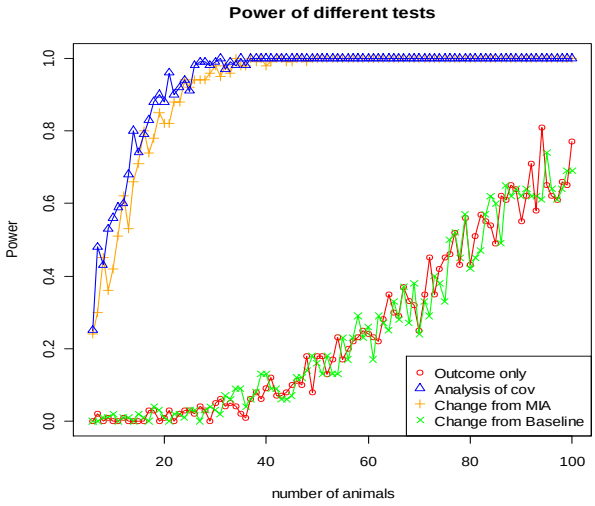

Figure 6: Power of different test in weak correlation condition. The circle line ○ is compare K_c and K_{veh} after treatment (a), while the cross line × means Change from K_b : $K_c - K_b$ versus $K_{veh} - K_b$ (b). The plus line + is Change from K_{mia} : $K_c - K_{mia}$ versus $K_{veh} - K_{mia}$ (c) and the triangular line △ is the linear regression model $K_c = a + b_1 K_{MIA} + b_2 Treatment$ (d)

Check whether all curves are monotonically increasing:

Table 8. Check monotonically of different test. Compare K_c and K_{veh} after treatment (a), Change from K_b (b), Change from K_{mia} (c) and linear regression model (d). 0 means the curve decrease and 1 means the curve increase.

Different test	a		b		c		d	
	0	1	0	1	0	1	0	1
	32	62	40	54	32	62	11	83

Table 8 shows that all curves are not monotonically increasing, which mean the power will not always increase when the number of observations increases.

We can get the conclusion from above result that the power of the linear regression is the largest one of these four results, the second is change from MIA point, the power of only comparing K_c after treatment is the smallest one.

4.42 No correlation:

For the assumption that there is no correlation between K_{mia} and K_b, we can just change step 2, which have been talked about before

Do the simulation again.

The simulation is same except step two, change step two to:

New step 2)

Assume that at the beginning of the research, the sum pressure of Left and right pawn T, which follows the normal distribution. $T \sim N(200,10)$. The proportion of the total weight put on the left pawn follows uniform distribution, $(0.2,0.3)$

This uniform distribution will generate small value, which means after MIA injection, it get pains and the pressure decrease. This change is same for both groups.

Table 9: Power of different tests in no correlation condition, a stands for compare K_c and K_{veh} after treatment, while b means Change from K_b : $K_c - K_b$ versus $K_{veh} - K_b$. C is Change from K_{mia} : $K_c - K_{mia}$ versus $K_{veh} - K_{mia}$ and d is the linear regression model $K_c = a + b_1 K_{MIA} + b_2 Treatment$. Pick up some of the powers to show power of a is always the smallest one, while the power of d is the largest one.

Power	**Different test**	**a**	**b**	**c**	**d**
animals' number					
6		0.19	0.07	0.3	0.35
10		0.36	0.17	0.55	0.58
20		0.66	0.33	0.96	0.97
30		0.82	0.44	1	1
40		0.9	0.49	1	1
50		0.93	0.5	1	1
60		0.99	0.58	1	1
70		1	0.78	1	1
80		1	0.74	1	1
90		0.99	0.82	1	1
100		1	0.84	1	1

Check whether all curves are monotonically increasing for the no correlation situation.

Table 10. Check monotonically of different test. Compare K_c and K_{veh} after treatment (a), Change from K_b (b), Change from K_{mia} (c) and linear regression model (d). 0 means the curve decrease and 1 means the curve increase.

Different test	a		b		c		d	
	0	1	0	1	0	1	0	1
	27	67	39	55	27	67	10	84

Table 9 shows that all curves are not monotonically increasing, which mean the power will not always increase when the number of observations increases.

We can get the conclusion from above result that the power of the linear regression is the largest one of these four results, the second is change from MIA point, the power of only comparing K_c after treatment is the smallest one.

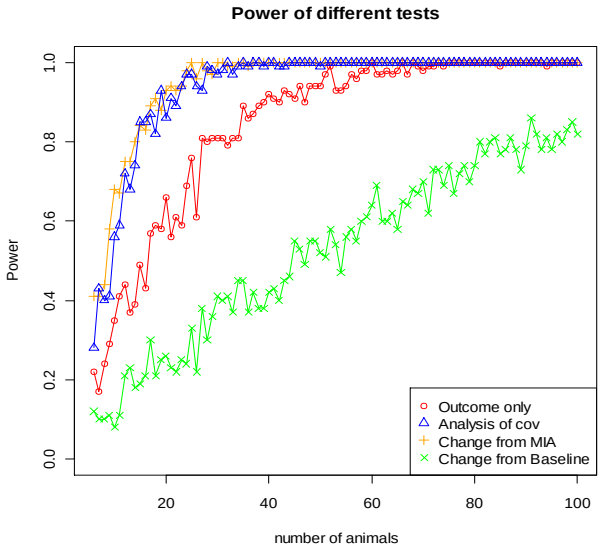

Figure 7: Power of different test in no correlation condition. The circle line ○ is compare K_c and K_{veh} after treatment (a), while the cross line × means Change from K_b : $K_c - K_b$ versus $K_{veh} - K_b$ (b). The plus line + is Change from K_{mia} : $K_c - K_{mia}$ versus $K_{veh} - K_{mia}$ (c) and the triangular line △ is the linear regression model $K_c = a + b_1 K_{MIA} + b_2 Treatment$ (d)

The similar conclusion can be draw from above results. The power of the linear regression is the largest one of these four results; the second is change from MIA point; the third is only comparing K_c after treatment, instead of the change from baseline.

5 Conclusion:

The aim to do this research is to find the best way to measure and analyze pain in animals.

In chapter 2 we described a typical experiment conducted to measure pain to find the best way to calculate the ratio. Compare M_A and M_B, two ways to calculate the ratio, M_A is better since it is unbiased and has lower variance. To prove this, we use both theoretical work and simulation.

In chapter 3, we focused on how to combine several measurements into one measurement of pain. There are several statistics for the measurement, such as L/R, R/L and $R-L$. One rule to choose is unbiased and the lowest coefficient of variation, all of these three is unbiased estimators, we choose L/R since it has the lowest coefficient of variation.

In chapter 4, we studied how to measure changes in pain. Four methods are listed:

a) Compare K_c and K_{veh} after treatment.

b) Change from K_b : $K_c - K_b$ versus $K_{veh} - K_b$

c) Change from K_{mia} : $K_c - K_{mia}$ versus $K_{veh} - K_{mia}$

d) Use the linear regression model $K_c = a + b_1 K_{MIA} + b_2 Treatment$

Compare the power of these tests; it is easy to find the best one is using the linear regression model $K_c = a + b_1 K_{MIA} + b_2 Treatment$.

If we cannot measure something, such as pain, happiness, or sadness, we can use indirect ways to calculate. The relationship should be obviously, or can be proved simple. Measure the pressure instead of pain support us a new view when we face to the similar difficult measurement in the future study.

6 Reference

1. Daigen X., Steven E R., Patsy C., Andre G., Bernard C., Sebastien G., Myriam S., Yves D., Richard W F., Nathalie M., Joseph M., Laurent A., Denis R.. 2008. MF63 [2-(6-Chloro-1*H*-phenanthro [9, 10-*d*] imidazol-2-yl)-isophthalonitrile], a Selective Microsomal Prostaglandin E Synthase-1 Inhibitor, Relieves Pyresis and Pain in Preclinical Models of Inflammation. *The journal of Pharmacology and Experimental Therapeutics. vol. 326, no. 3 754-763*
2. Stephen S., 2008. Statistical Issues in Drug Development. *John wiley & sons*. P.95-106
3. G.M.P. van K. and LJ. Van V., 1999. Mean and variance of Ratio Estimators Used in Fluorescence Ratio Imaging. *Wiley-Liss, Inc*, Cytometry 39: 300-305.
4. Jeong S.H., Gary C. B., Weidong L., Justina J., Volker N.. Computerized analysis of audible and ultrasonic vocalizations of rats as a standardized measure of pain-related behavior. 2004. *Journal of Neuroscience Method, 141(2005), p.261-269.*

7 Appendix

Figure 1:

```
rm(list=ls())

h=numeric()
m=numeric()
p=numeric()
q=numeric()

for (t in seq(1,1001,10))
{
for (i in 1:100)
{
for (j in 1:100)
{
a=rnorm(t,200,30)
b=runif(t,0.25,0.4)
x=a*b
y=a*(1-b)
k=sum(x)/sum(y)
l=mean(x/y)
}
h=c(h,k)
m=c(m,l)
}
mean(h)
mean(m)
p=c(p,mean(h))
q=c(q,mean(m))
}
truevalue=((0.25+0.4)/2)/(1-(0.25+0.4)/2)
plot(seq(1,1001,10),p,ylim=c(0.48,0.49),pch=1,col="red",xlab="n",ylab="ratio
mean",main="Compare MA and MB")
points(seq(1,1001,10),q,pch=2,col="blue")
lines(seq(1,1001,10),p,col="red")
lines(seq(1,1001,10),q,col="blue")
abline(h=truevalue,col="green")
leg.txt<- c("MA","MB","True Value")
leg.col<-c("red","blue","green")
leg.pch<-c(1,2,".")
legend("right",leg.txt,col=leg.col,pch=leg.pch)
```

Figure 2:

```
rm(list=ls())

h=numeric()
m=numeric()
p=numeric()
q=numeric()

for (t in seq(1,1001,10))
{
for (i in 1:100)
{
for (j in 1:100)
{
a=rnorm(t,200,30)
b=runif(t,0.25,0.4)
x=a*b
y=a*(1-b)
k=sum(x)/sum(y)
l=mean(x/y)
}
h=c(h,k)
m=c(m,l)
}
var(h)
var(m)
p=c(p,var(h))
q=c(q,var(m))
}
plot(seq(1,1001,10),p,pch=1,,col="red",xlab="n",ylab="ratio variance",main="variance of MA and MB")
points(seq(1,1001,10),q,pch=2,col="blue")
lines(seq(1,1001,10),p,col="red")
lines(seq(1,1001,10),q,col="blue")
leg.txt<- c("MA","MB")
leg.col<-c("red","blue")
leg.pch<-c(1,2)
legend("topright",leg.txt,col=leg.col,pch=leg.pch)
```

Figure 3:

```
rm(list=ls())
h=numeric()
m=numeric()
p=numeric()
q=numeric()

for (t in seq(1,1000,10))
{
for (i in 1:100)
{
for (j in 1:100)
{
a=rnorm(t,200,30)
b=runif(t,0.25,0.4)
x=a*b
y=a*(1-b)
k=sum(y)/sum(x)
l=mean(y/x)
}
h=c(h,k)
m=c(m,l)
}
mean(h)
mean(m)
p=c(p,mean(h))
q=c(q,mean(m))
}
truevalue=(1-(0.25+0.4)/2)/((0.25+0.4)/2)
plot(seq(1,1000,10),p,ylim=c(2.07,2.14),pch=1,col="red",xlab="n",ylab="ratio",main="alternative
estimator 1: R/L")
points(seq(1,1000,10),q,pch=2,col="blue")
lines(seq(1,1000,10),p,col="red")
lines(seq(1,1000,10),q,col="blue")
abline(h=truevalue,col="green")
leg.txt<- c("MC","MD","True Value")
leg.col<-c("red","blue","green")
leg.pch<-c(1,2,".")
legend("right",leg.txt,col=leg.col,pch=leg.pch)
```

Figure 4:

```
rm(list=ls())

h=numeric()
p=numeric()

for (t in seq(1,1000,10))
{
for (i in 1:100)
{
for (j in 1:100)
{
a=rnorm(t,200,30)
b=runif(t,0.25,0.4)
x=a*b
y=a*(1-b)
k=y-x
}
h=c(h,k)
}
mean(h)
p=c(p,mean(h))
}
truevalue=200*(1-(0.25+0.4))
plot(seq(1,1000,10),p,pch=1,ylim=c(69.5,71),col="red",xlab="n",ylab="ratio",main="alternative
estimator 2: R-L")
lines(seq(1,1000,10),p,col="red")
abline(h=truevalue,col="green")
leg.txt<- c("R-L","True Value")
leg.col<-c("red","green")
leg.pch<-c(1,5)
legend("topright",leg.txt,col=leg.col,pch=leg.pch)
```

Figure 6&7

```
rm(list=ls())
res1=res2=res3=plot1=plot2=plot3=numeric()
for (t in 6:100){
for (i in 1:100){
for (j in 1:100){
xc=rnorm(t,200,40)
r1=runif(t,0.45,0.55)
c1=xc*r1
c2=xc*(1-r1)
k1=c1/c2
xv=rnorm(t,200,40)
r2=runif(t,0.45,0.55)
v1=xv*r2
v2=xv*(1-r2)
m1=v1/v2
#generate mia(correlate)
r3=runif(t,0.1,0.7)
cm=k1*r3
vm=m1*r3
#generate treatment
r5=runif(t,0.95,1.6)
r6=runif(t,0.9,1.3)
c3=cm*r5
v3=vm*r6}
res1[i]=t.test(c3,v3)$p.value
res2[i]=t.test(c3-k1,v3-m1)$p.value
res3[i]=t.test(c3-cm,v3-vm)$p.value}
plot1[t]=mean(ifelse(res1<0.05,1,0))
plot2[t]=mean(ifelse(res2<0.05,1,0))
plot3[t]=mean(ifelse(res3<0.05,1,0))}
c(plot1[6],plot1[10],plot1[20],plot1[30],plot1[40],plot1[50],plot1[60],plot1[70],plot1[80],plot1[90
],plot1[100])
c(plot2[6],plot2[10],plot2[20],plot2[30],plot2[40],plot2[50],plot2[60],plot2[70],plot2[80],plot2[90
],plot2[100])
c(plot3[6],plot3[10],plot3[20],plot3[30],plot3[40],plot3[50],plot3[60],plot3[70],plot3[80],plot3[90
],plot3[100])
plot(6:100,plot1[6:100],pch=1,ylim=c(0,1),xlab="number                                    of
animals",ylab="Power",main="Power of different tests",col="red")
points(6:100,plot2[6:100],pch=4,col="green")
points(6:100,plot3[6:100],pch=3,col="orange")
lines(plot1,col="red")
lines(plot2,col="green")
lines(plot3,col="orange")
```

```
rm(list=ls())
pv=numeric()
plot4=numeric()

for (l in 6:100)
{
for (i in 1:100)
{
xc=rnorm(l,200,40)
r1=runif(l,0.45,0.55)
c1=xc*r1
c2=xc*(1-r1)
k1=c1/c2

xv=rnorm(l,200,40)
r2=runif(l,0.45,0.55)
v1=xv*r2
v2=xv*(1-r2)
m1=v1/v2

r3=runif(l,0.1,0.5)
cm=k1*r3
vm=m1*r3

r5=runif(l,0.95,1.6)
r6=runif(l,0.9,1.3)
c3=cm*r5
v3=vm*r6

t=c(rep(0,l),rep(1,l))
l1=lm(c(c3,v3)~c(cm,vm)+t)
pv[i]=summary(l1)[[4]][12]
}
plot4[l]=mean(ifelse(pv<0.05,1,0))
}
points(6:100,plot4[6:100],pch=2,col="blue")
c(plot4[6],plot4[10],plot4[20],plot4[30],plot4[40],plot4[50],plot4[60],plot4[70],plot4[80],plot4[90
],plot4[100])
lines(plot4,col="blue")
leg.txt<- c("Outcome only","Analysis of oov","Change from Baseline","Change from MIA")
leg.col<-c("red","blue","orange","green")
leg.pch<-c(1,2,3,4)
legend("bottomright",leg.txt,col=leg.col,pch=leg.pch):
```

Figure 8:

```
rm(list=ls())
varc= varcb= varcm= plot1= plot2= plot3= plot4=numeric()
for (i in 1:10){
for (j in 1:100){
xc=rnorm(100,200,40)
r1=runif(100,0.45,0.55)
c1=xc*r1
c2=xc*(1-r1)
k1=c1/c2
xv=rnorm(100,200,40)
r2=runif(100,0.45,0.55)
v1=xv*r2
v2=xv*(1-r2)
m1=v1/v2
#generate mia(correlate)
r3=runif(100,0.1+0.025*i,0.7-0.025*i)
cm=k1*r3
vm=m1*r3
#generate treatment
r5=runif(100,0.95,1.6)
r6=runif(100,0.9,1.3)
c3=cm*r5
v3=vm*r6
t=c(rep(1,100),rep(0,100))
l1=lm(c(c3,v3)~c(cm,vm)+t)
vc=var(c3-v3)
vcb=var((c3-k1)-(v3-m1))
vcm=var((c3-cm)-(v3-vm))
vl=var(c(c3,v3))}
varc[i]=vc
varcb[i]=varc
varcm[i]=vcm
varl[i]=vl}
plot(seq(0,1,length = length(varc)),varc,xlim=c(0,1),ylim=c(0,0.05),pch=1,xlab="Correlation coefficient",ylab="Variance",main="Variance change when correlatioin coefficient change",col="red")
points(seq(0,1,length = length(varcb)),varcb,pch=4,col="green")
points(seq(0,1,length = length(varcm)),varcm,pch=3,col="orange")
points(seq(0,1,length = length(varl)),varl,pch=2,col="blue")
lines(seq(0,1,length=length(varc)),varc,col="red")
lines(seq(0,1,length=length(varcb)),varcb,col="green")
lines(seq(0,1,length=length(varcm)),varcm,col="orange")
lines(seq(0,1,length=length(varl)),varl,col="blue")
```

```
leg.txt<- c("Outcome only","Analysis of oov","Change from Baseline","Change from MIA")
leg.col<-c("red","blue","orange","green")
leg.pch<-c(1,2,3,4)
legend("topright",leg.txt,col=leg.col,pch=leg.p
```