

## **Sample sizes for clinical trials using sputum eosinophils as a primary outcome**

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## **ABSTRACT**

Clinical trials do not report sputum eosinophil data in a consistent method and this makes it difficult to compare across studies and to evaluate the sample sizes estimated in these studies. The objectives of the paper are 1) to systemically review reporting effect size and sample calculations in randomized controlled trials using sputum eosinophil count as a primary outcome and 2) to illustrate sample size estimation under different methods of data representation using data from an effective anti-eosinophil treatment strategy (mepolizumab). RCTs in adults (excluding allergen provocation models) of treatment of asthma and COPD for the past 10 years were searched in OVID MEDLINE and 20 studies were identified that met all the inclusion criteria. Only 9 studies discussed sample size calculation. Change from baseline was used as an outcome in 11 studies and was expressed as change in absolute percentage count, percentage change from baseline or as fold changes. Assuming a minimal clinically important reduction of 15% in absolute terms, 18 subjects in each arm will be required to achieve 80% power using a ANCOVA analysis that we recommend to detect a significance with an alpha error of 0.05.

## INTRODUCTION

Eosinophil in sputum is a valid and reliable outcome measure in clinical trials of patients with eosinophilic lung diseases like asthma <sup>1, 2</sup>. Clinical trials of corticosteroids and specific anti-eosinophil agents like mepolizumab have consistently shown a reduction in sputum eosinophil counts <sup>3,4,5,6</sup>. The recent guidelines for clinical end points in asthma trials set out by the American Thoracic Society (ATS) and the European Respiratory Society (ERS) have also incorporated the use of induced sputum eosinophil counts as an outcome measure <sup>7</sup>. This document however does not provide guidelines for expressing results or calculating sample sizes for clinical trials which use sputum eosinophils as an outcome.

Clinical trials with two groups and with pre and post interventional data in each group usually compare either the post scores or change from baseline scores using either a T –test or an analysis of covariance adjusting for baseline differences. The latter method of analysis has been held to be superior to all other methods by statisticians as it has the highest statistical power <sup>8</sup>. For sputum eosinophils, which are conventionally expressed as a percentage of the total cell count, change from baseline is preferred over post scores as it is clinically more meaningful and gives an estimate of the amount of improvement or deterioration caused by an intervention. It has been observed that clinical trials reporting change from baseline of sputum eosinophils counts have expressed results as fold changes (ratio of pre and post scores), percentage from baseline or as change in absolute percentage counts. Although none of the three are superior to one another from a biological point of view, change from baseline in terms of absolute percentage count is preferable due to its better clinical interpretability and lack of need for statistical data manipulation. Expressing results as fold changes or percentage change from baseline may be

challenging especially when there are zero counts as often happens in clinical practice. These methods also convert the values into ratios which therefore have a non-Normal or skewed distribution necessitating data transformation (usually log) prior to carrying out statistical tests that assumes Normal distribution. The zero values are usually either replaced by positive number like 0.1 or 0.001<sup>9</sup> or a constant number e.g. 0.2<sup>7</sup> added prior to log transformation.

Consequent to the differences in expressing results, sample size estimation methods vary significantly in these clinical trials as sample sizes depend on the selected effect sizes and the standard deviation of the primary outcome variable <sup>10</sup>. Often sample size reporting is incomplete too. We are not aware of any study so far that has examined the completeness or the quality of reporting sample sizes calculations, effect sizes, analysis methods or demonstrated the method of doing so for clinical trials using change in sputum eosinophil count from baseline as the primary outcome .

We therefore decided to 1) systematically review current practice of reporting effect sizes and sample size calculations in randomized controlled trials using sputum eosinophil count as a primary outcome; 2) describe and illustrate how to apply different strategies for defining outcomes using sputum eosinophil count in interventional studies that have both baseline and post-intervention data; and 3) demonstrate sample size estimation under the different method of data representation illustrated in 2) and data analysis with a dataset from a prior clinical trial <sup>3</sup>.

## **METHODS**

### **Search Strategy**

The OVID search engine was used to search for all randomized controlled trials (RCTs) in English language in the past 10 years with “asthma” AND “sputum eosinophils” OR “airway

inflammation” as key search words and using the OVID MEDLINE(R), Embase and OVID Healthstar databases. The inclusion criteria of the articles were 1) studies on asthma and chronic obstructive pulmonary diseases (COPD); 2) RCTs involving drug trials on human subjects; and 3) studies using sputum eosinophil measures as a primary outcome. Both parallel group and cross over studies were included. Studies were included if the primary aim was to look at airway inflammation even if sputum eosinophil was not the only primary outcome. Studies involving children, allergen or antigen provocation models, environmental exposure studies and those using nonparametric tests as the primary methods of analysis were excluded. Data were extracted independently by two reviewers (AD and WZ) after going through the methods section of all the studies and tabulated carefully using a data abstraction form . Any disagreement between the two reviewers were discussed and resolved by consensus.

### **Different strategies for defining outcomes**

Sputum eosinophils are customarily expressed as percentages of total cell counts and not as absolute counts <sup>11</sup>. Change from baseline for sputum eosinophils for asthma and COPD trials can be represented in three different ways as follows:

- 1) Change from baseline in absolute percentage counts = pre% - post% (or post% - pre%)
- 2) Fold Changes = pre%/post% (or post%/pre %)
- 3) Percentage change from baseline = (pre% - post%) \* 100 / pre %

Data from the mepolizumab study<sup>3</sup> was used to demonstrate the above strategies. Zero values were replaced by 0.1 prior to all calculations .

### **Demonstration of sample size calculation**

Sample size calculations were demonstrated using the above dataset for independent parallel group superiority studies for t-tests to achieve 80% power and a Type I error of 0.05 assuming equal sample sizes in intervention and placebo group. The standard deviations and the effect sizes for change in absolute percent counts were calculated. Fold changes were log transformed (natural logs) and zero counts replaced by 0.1 prior to transformation. We also used the data to calculate the sample size with fold changes as outcome. For this calculation, both standard deviation and effect estimate were on log scale.

The sample size formula used was <sup>10</sup>

$$n = 2 \times s^2 \times \frac{(Z_{\alpha/2} + Z_{1-\beta})^2}{\delta_0^2} \dots\dots\dots \text{(Equation 1)}$$

Where n=size per group;  $Z_{\alpha/2}$  is the standard Normal z-score corresponding to the two-sided  $\alpha$  level of significance;  $Z_{1-\beta}$  is the standard Normal z-score corresponding to the probability  $\beta$  of type II error;  $\delta_0$ = the minimum clinically important difference; s = prior estimate of the pooled standard deviation of the estimate of the difference between groups.

Sample size calculation using analysis of covariance (ANCOVA) was done for a hypothetical superiority trial where the clinically minimal important difference was 15% in absolute percent change or 2 fold-change difference. Sample size of ANCOVA was calculated from that of the T-test while adjusting by a factor of  $(1-\rho^2)$  where  $\rho$  is the correlation between the baseline and the final outcome measure<sup>12</sup>

## RESULTS

### Reporting of Sample size calculation

Of the 361 search results, 20 studies fulfilled the inclusion criteria<sup>9, 15-34</sup>. 11 of the 20 (55%) did not report how the sample size was calculated. Of the 9 other articles that reported sample size calculation, only 2 articles had detailed all the required parameters related to sample size calculation and only one article contained all 8 items within the checklist (Table 1A). The articles expressed outcome variably as change in absolute percentage counts, percentage changes from baseline or as fold changes. One article reported as both change in absolute percentage count and as percentage change from baseline. Nine articles reported post score comparison while the rest used change from baseline data for analysis. (Table 1B). Six articles used ANCOVA for statistical analysis but none of the studies adjusted sample sizes for this.

### **Demonstration of strategies for defining outcomes**

The mepolizumab trial was a parallel group randomized control trial with 10 patients in the placebo group and 8 patients in the intervention (mepolizumab) group with pre and post intervention sputum eosinophil counts. The raw data for each of the groups and the different ways of expressing change from baseline are demonstrated below (Table 2A and B). In general, changes in absolute percent counts, being the difference between pre and post values, may be assumed to be Normally distributed despite the distribution of the pre and post values being non-Normal. On the other hand, fold changes being ratios have a skewed statistical distribution. Hence fold changes were log transformed prior to calculating the standard deviation and effect estimate. Similar results for percentage change from baseline were expressed as median (minimum, maximum).

In the placebo group sputum eosinophils increased by 17.9% (standard deviation of 27.8%) while in the intervention group it decreased by 21.05% (standard deviation of 14.6%). The effect size was therefore 38.9 % (absolute percent counts) or 54 fold (or 3.8 in log scale). The pooled standard deviation was 23% and 1.47 in absolute percent counts and log (fold changes) respectively. The median change in terms of percentage change from baseline was -251.2% (-1669.4%, 60.6%) for the placebo group and 93.8% (71.9%, 99.6%) for the intervention group.

### **Demonstration of sample size calculation**

The values of standard deviation and effect sizes expressed as change in absolute percentage counts and log transformed fold changes were plugged into equation 1 and calculations made for sample sizes (Table 3). From the mepolizumab clinical trial data the correlation between pre and change from baseline in absolute percentage counts and in the log scale is -0.73 and 0.72 respectively. Therefore the number of participants required will be reduced by a factor of 0.47. Thus 1 (2 X 0.47) patients in each arm or 3 (6 X 0.47) patients in each arm will be sufficient when outcomes are expressed as change in absolute percentage counts and fold changes respectively, other parameters remaining constant.

## **DISCUSSION**

### **Reporting sample sizes and effect sizes**

Sample size calculations are underreported in clinical drug trials using sputum eosinophils as a primary outcome. This is clear from the results of our systematic review. This reduces the accuracy of trials and leads to inappropriate conclusions. There are also substantial variations in

the way change from baseline of sputum eosinophils is expressed which makes comparison between clinical trials and drug effects difficult. There is thus an unmet need to formulate guidelines for reporting drug effects when using sputum eosinophil counts in clinical trials. We did not examine the differences in sample size estimations and reporting between asthma and COPD trials as the variance of change of sputum eosinophils and the minimal clinically important difference are conventionally taken to be the same for both diseases. Therefore sample size estimation is unlikely to be affected by the disease definition. We also did not include studies in subjects with mild intermittent asthma where effects of various interventions in attenuating allergen-induced eosinophilic inflammation were examined. This was for two reasons, First, we wanted to limit our estimation of sample sizes to clinical trials that evaluated therapies that are relevant to patients with mild persistent or moderate to severe asthma in phase IIb or III studies rather than experimental therapies in subjects with mild intermittent asthma, Second and more importantly, mechanisms of reversal of established eosinophilia may be very different to those that prevent recruitment of eosinophils (such as following an allergen inhalation) and therefore effect sizes for these two separate interventions may be different. This needs to be investigated in a separate study,

### **Expressing Change from Baseline**

Of the three methods of expressing change from baseline, percentage change from baseline has been considered to be an inefficient method by statisticians as it does not correct for imbalance between groups at baseline and has a non-Normal distribution<sup>8</sup>. Further when used in sputum eosinophil trials, as demonstrated with this data set, percentage change from baseline values may be both negative and positive and also numerically very large. This makes data transformation

difficult and percentage change from baseline is therefore not a very useful method of data representation for sputum eosinophil studies.

On the other hand, change in absolute percentage count has greater clinical interpretability compared to fold changes as exemplified below. A change from 25% to 5% is a 20% change in absolute percent counts which is equivalent to a 5 fold change or 80% change from baseline. Again a reduction from 5 to 1% is a 4% change in absolute percent counts but has the same fold and percentage changes as the former case. In the latter case the sputum eosinophils have been normalized and a patient with such a report will not require additional treatment. But in the former situation a patient needs to be treated further to reduce the counts into the normal range. In another example it may be shown that a change from 25% to 0.1% is a 250 fold change which is the same fold change as a change from 5% to 0.2%. It is obvious that the former change has more clinical significance than the latter. Therefore, it may be reasonable to suggest that effect sizes or outcomes in clinical trials be expressed in terms of absolute percentage counts for better interpretation of results in the clinical context. The use of fold changes may be justified for use only in those situations where data transformation is needed for statistical purposes. In clinical practice and in dose-response studies<sup>34</sup>, it is generally observed that sputum eosinophil percentages change proportional to the baseline values although this has not been evaluated systematically.

### **Sample size calculation**

Sample size calculations using the mepolizumab data show that only 6 or less subjects in each arm in a parallel group randomized study on severe asthma subjects may have sufficient power to detect significant changes when using a t-test assuming that all participants have the same

baseline risk. The reason for such small sample size estimation is the large effect size of this trial. Such large effect sizes are however not unusual in severe asthma trials using anti eosinophil specific therapies <sup>4</sup>. Corticosteroids have shown a reduction in the range of two- to seven folds <sup>13, 14</sup> or 15% to 20% in absolute sputum eosinophil percentages in clinical trials of severe eosinophilic asthma <sup>9, 15</sup>. Smaller effect sizes are however seen with milder asthma trials <sup>16</sup>.

The standard minimal clinically important difference (MCID) for sputum eosinophil studies has been traditionally accepted to be 2 fold or 50% change <sup>7</sup>. From Figure 2, which shows the sample size estimates for various effect sizes, it may be appreciated that clinical trials such as the mepolizumab study will have 80% power for detecting such a change with 72 participants in each arm. A consensus agreement on the MCID in terms of absolute percent counts has not yet been reached. However, as mentioned earlier corticosteroids have shown a reduction of eosinophil counts between 15% to 20% in various severe asthma studies. Therefore it may be reasonable to assume an MCID of 15% in terms of absolute percentage counts when performing severe asthma trials with baseline eosinophil counts of more than 15%. Thus 37 and 18 subjects in each arm will be required to achieve a 80% power using a t-test and ANCOVA respectively..

It may also be seen that the estimate using log transformation (in foldchange calculations) is much smaller than that using absolute percent counts. This is because the variance gets stabilized as a result of the transformation, thereby reducing the sample size. Thus sample size estimates are also somewhat affected by the method of data representation. From our calculations there was a reduction in sample size estimates by a factor of 2 when similar sample size parameters were used. However, due to the increased level of complexity when using ANCOVA, the gained statistical power is often out-balanced by the difficulty in employing the method.

## **CONCLUSION**

Clinical trials using sputum eosinophils as a primary outcome do not often report how or the parameters assumed for sample size calculations. Sample sizes in these trials are affected not only by method of analysis, study design, MCID and variance but also by strategies adopted for data representation for defining outcomes. The best method to adopt for such trials is to express results as change in absolute percentage counts as this is clinically most relevant and does not involve statistical intricacies of data transformation. Adjusting calculated sample sizes based on use of ANCOVA may reduce the sample size estimates by half. A sample size of 18 patients in each arm in an ANCOVA superiority trial may be sufficient to achieve an 80% power with an alpha 0.05 to detect a 15% difference in absolute percentage counts in severe asthma studies with specific anti-eosinophil agents such as the mepolizumab.

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**Figure 1: Study selection. The data in this figure refer to the search completed in March 15th, 2012**

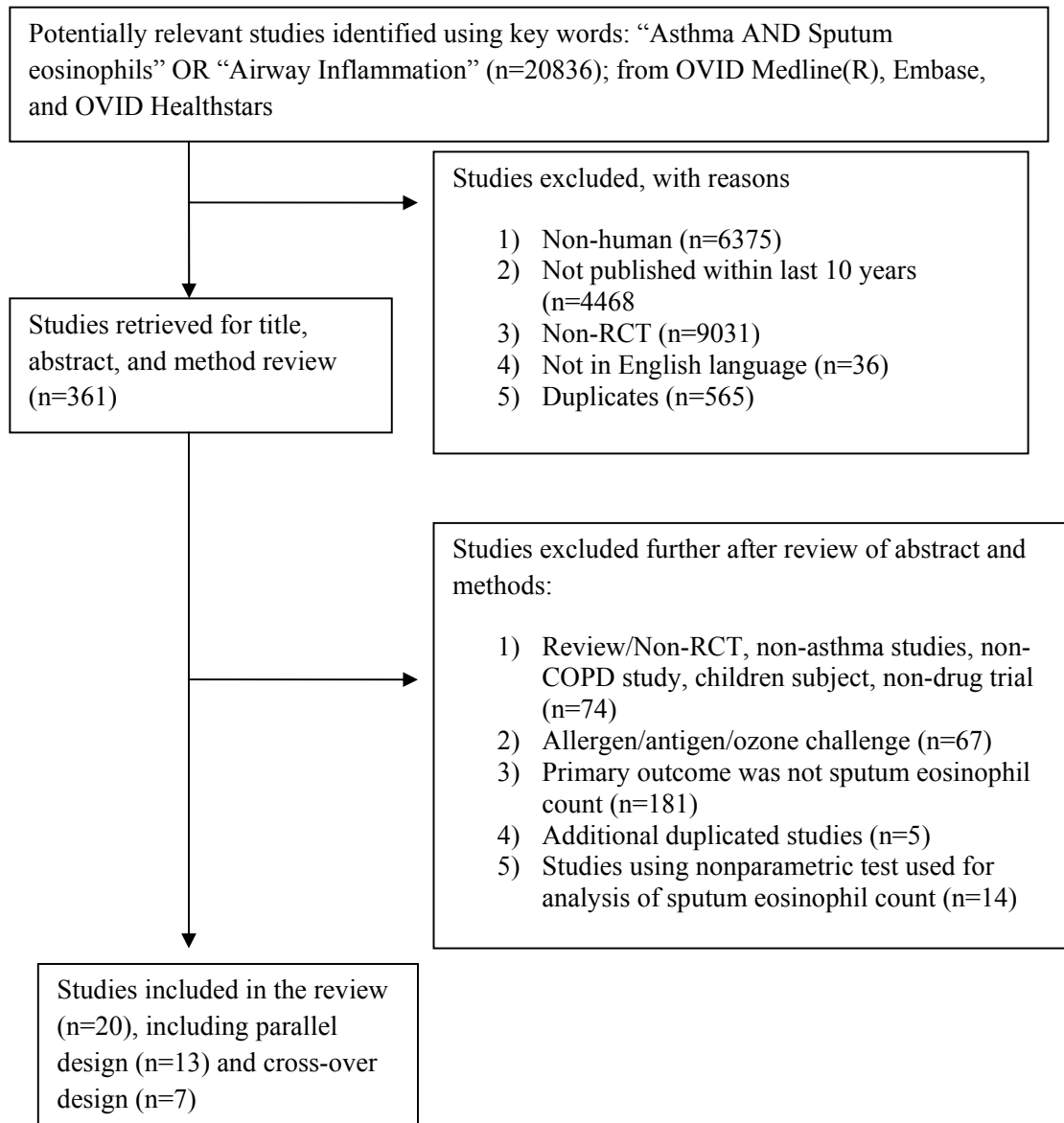


**Table 1A: Details of method used for effect representation and samples sizes for included studies (n=20)**

|    | <b>Author</b>                        | <b>Journal and year of publication</b>                        | <b>Method of data representation</b>                                | <b>Type of Analysis for between groups</b>                     | <b>Total Sample size</b> | <b>Study Design</b> |
|----|--------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------------|----------------------------------------------------------------|--------------------------|---------------------|
| 1  | Green, RH et al <sup>16</sup>        | Lancet 2002                                                   | Log-transformed, Percentage difference between groups               | Post score comparison by Student's T test                      | 74                       | Parallel            |
| 2  | Kanniess, F et al <sup>17</sup>      | European Respiratory Journal 2002                             | Fold changes                                                        | Change from baseline by analysis of variance                   | 40                       | Crossover           |
| 3  | Minoguchi, K et al <sup>18</sup>     | Chest 2002                                                    | Abs% count                                                          | Change from baseline by analysis of variance                   | 29                       | Crossover           |
| 4  | Urata, Y et al <sup>19</sup>         | Respiratory Medicine 2002                                     | Abs% counts                                                         | Post score comparison by Student's T test                      | 33                       | Crossover           |
| 5  | Yoshida, M et al <sup>20</sup>       | Journal of Asthma 2002                                        | Abs% counts                                                         | Post score comparison by Student's T-test                      | 28                       | Parallel            |
| 6  | Grootendorst, DC et al <sup>21</sup> | Pulmonary Pharmacology & Therapeutics 2003                    | Log-transformed                                                     | Post scores comparison by analysis of variance                 | 18                       | Crossover           |
| 7  | Djukanovic, R et al <sup>22</sup>    | American Journal of Respiratory & Critical Care Medicine 2004 | Abs% counts                                                         | Post score comparison by analysis of covariance                | 45                       | Parallel            |
| 8  | Kobayashi, M et al <sup>23</sup>     | Respirology 2004                                              | % change from baseline                                              | Change from baseline by repeated measures analysis of variance | 11                       | Parallel            |
| 9  | Ten Brinke, A et al <sup>24</sup>    | American Journal of Respiratory & Critical Care Medicine 2004 | Log-transformed % count                                             | Post score comparison by analysis of covariance                | 22                       | Parallel            |
| 10 | Jayram, L et al <sup>25</sup>        | European Respiratory Journal 2005                             | Log transformed, Percentage difference in treatment between groups. | Post scores comparison by analysis of covariance               | 14                       | Crossover           |
| 11 | Jayaram, L et al <sup>15</sup>       | Thorax 2005                                                   | Log-transformed, Abs% counts and % change from baseline             | Change from baseline by analysis of covariance                 | 50                       | Crossover           |
| 12 | Green, RH et al <sup>26</sup>        | European Respiratory Journal 2006                             | Log-transformed, fold change from baseline                          | Change from baseline by two-way analysis of variance           | 49                       | Crossover           |
| 13 | Hauber, H et al <sup>27</sup>        | Canadian Respiratory                                          | Abs % counts                                                        | Change from baseline by analysis of variance                   | 17                       | Crossover           |

|    |                                   |                                               |                                            |                                                                              |     |           |
|----|-----------------------------------|-----------------------------------------------|--------------------------------------------|------------------------------------------------------------------------------|-----|-----------|
|    |                                   | Journal 2006                                  |                                            |                                                                              |     |           |
| 14 | Haverkamp, HC et al <sup>28</sup> | Journal of Allergy & Clinical Immunology 2007 | Abs% counts                                | Change from baseline by unpaired Student's T test with Bonferroni correction | 19  | Parallel  |
| 15 | Pavord, I et al <sup>29</sup>     | Respiratory Research 2007                     | Abs % count                                | Change from baseline by analysis of covariance                               | 66  | Parallel  |
| 16 | Chen, YH et al <sup>30</sup>      | Pulmonary Pharmacology & Therapeutics 2008    | Abs% counts                                | Post score comparison by unpaired Student's T test                           | 37  | Parallel  |
| 17 | Duong, M et al <sup>31</sup>      | Chest 2008                                    | Log-transformed                            | Change from baseline by analysis of variance                                 | 26  | Parallel  |
| 18 | Hoshino, M et al <sup>32</sup>    | Allergology International 2009                | Abs% counts                                | Change from baseline by two sample T test and Mann Whitney U test            | 27  | Crossover |
| 19 | Pavord, ID et al <sup>33</sup>    | Journal of Allergy & Clinical Immunology 2009 | Log transformed, Fold change from baseline | Change from baseline by analysis of covariance                               | 127 | Parallel  |
| 20 | Dente, FL et al <sup>9</sup>      | Annals of Allergy, Asthma, & Immunology 2010  | Log-transformed                            | Post score comparison by Analysis of variance                                | 59  | Parallel  |

**Abs% counts: absolute percent counts; % change from baseline:percentage change from baseline**

**Table 1B: Results of systematic review**

**(Within bracket figures denote % of total number of papers extracted for analysis; n=20)**

| <b>Item Number</b> | <b>Descriptor</b>                                                                 | <b>Yes (%)</b> | <b>No (%)</b> | <b>Unclear (%)</b> |
|--------------------|-----------------------------------------------------------------------------------|----------------|---------------|--------------------|
| 1.                 | Desired Significance level                                                        | 8(40%)         | 12(60%)       | 0                  |
| 2.                 | Desired power level                                                               | 7(35%)         | 13(65%)       | 0                  |
| 3.                 | Type of test statistics of method of analysis for the primary outcome             | 7(35%)         | 11(55%)       | 2(10%)             |
| 4.                 | Description of the primary outcome (absolute count, log-transformed, fold change) | 20(100%)       | 0             | 0                  |
| 5.                 | Minimal clinically important difference                                           | 7(35%)         | 13(65%)       | 0                  |
| 6.                 | Variance for sample size estimation                                               | 2(10%)         | 18(90%)       | 0                  |
| 7.                 | Calculated Sample size                                                            | 7(35%)         | 13(65%)       | 0                  |
| 8.                 | Software/reference/formula for sample size calculation provided                   | 1(5%)          | 17(85%)       | 2(10%)             |

**Table 2A: The Placebo Group**

| Pt ID              | Pre  | Post | Change from baseline of % counts (post-pre) | Fold Changes (pre/post) | Percentage from baseline | Natural Log of fold changes |
|--------------------|------|------|---------------------------------------------|-------------------------|--------------------------|-----------------------------|
| 1                  | 3.7  | 18.7 | 15.0                                        | 0.19                    | - 405.4                  | -1.62                       |
| 2                  | 3.6  | 63.7 | 60.1                                        | 0.05                    | -1669.4                  | -2.87                       |
| 3                  | 4.7  | 62.3 | 57.6                                        | 0.07                    | -1225.5                  | -2.58                       |
| 4                  | 4.5  | 25.3 | 20.8                                        | 0.17                    | -462.2                   | -1.72                       |
| 5                  | 4.0  | 5.0  | 1.0                                         | 0.8                     | -25                      | -0.22                       |
| 6                  | 35.3 | 15.5 | -19.8                                       | 2.27                    | 56.1                     | 0.82                        |
| 7                  | 3.3  | 1.3  | -2.8                                        | 2.53                    | 60.6                     | 0.93                        |
| 8                  | 6.0  | 5.0  | -1.0                                        | 1.2                     | 16.6                     | 0.18                        |
| 9                  | 4.6  | 5.3  | 0.7                                         | 0.86                    | -15.2                    | -0.14                       |
| 10                 | 3.2  | 50.0 | 46.8                                        | 0.064                   | -1462.5                  | -2.74                       |
| Mean               |      |      | 17.9                                        |                         | -215.2(median)           | -1.0                        |
| Standard deviation |      |      | 27.8                                        |                         |                          | 1.48                        |

**Table 2B: The Mepolizumab (Intervention) Group**

| Pt ID              | Pre  | Post                | Change from baseline of % counts(post-pre) | Fold changes (pre/post) | Percentage from baseline | Natural Log of fold changes |
|--------------------|------|---------------------|--------------------------------------------|-------------------------|--------------------------|-----------------------------|
| 1                  | 21.0 | 1.3                 | -19.7                                      | 16.15                   | 93.8                     | 2.78                        |
| 2                  | 25.5 | 0.1<br>(0 replaced) | -25.4                                      | 255                     | 99.6                     | 5.54                        |
| 3                  | 54.3 | 11.3                | -43.0                                      | 4.80                    | 79.1                     | 1.57                        |
| 4                  | 16.6 | 1.0                 | -15.6                                      | 16.6                    | 93.9                     | 2.81                        |
| 5                  | 42.3 | 0.6                 | -41.7                                      | 70.5                    | 98.5                     | 4.26                        |
| 6                  | 10.7 | 3.0                 | -7.7                                       | 3.57                    | 71.9                     | 1.27                        |
| 7                  | 6.0  | 0.3                 | -5.7                                       | 20                      | 9.5                      | 2.99                        |
| 8                  | 12.3 | 2.7                 | -9.6                                       | 4.56                    | 78.4                     | 1.52                        |
| Mean               |      |                     | -21.1                                      |                         | 93.8(median)             | 2.8                         |
| Standard deviation |      |                     | 14.6                                       |                         |                          | 1.47                        |

**Table 3: Sample sizes for various representations of data using t-test**

| Representation of data                                                        | Means<br>Placebo (n=10)<br>Vs<br>Mepolizumab<br>(n=8) | Measures of<br>dispersion                            | Diff<br>between<br>the groups<br>detected or<br>relative<br>effect | Sample<br>sizes for<br>each<br>group |
|-------------------------------------------------------------------------------|-------------------------------------------------------|------------------------------------------------------|--------------------------------------------------------------------|--------------------------------------|
| Change in absolute percent<br>count<br><br>(baseline% - end of<br>treatment%) | 17.92%<br><br>vs<br><br>-21.05                        | Pooled SD<br><br>= 22.9 %                            | 38.9%                                                              | 6                                    |
| Mean fold change<br><br>(baseline/end of treatment)                           | 0.9 fold<br><br>Vs<br><br>48.9 fold                   | Pooled SD<br>(in natural<br>log scale)<br><br>= 1.47 | 54 fold or<br>3.8 (ln<br>scale)                                    | 2                                    |

SD = standard deviation; ln= natural log

**Figure 2: Sample sizes for demonstrating a range fold changes in eosinophil percent between active and placebo group**

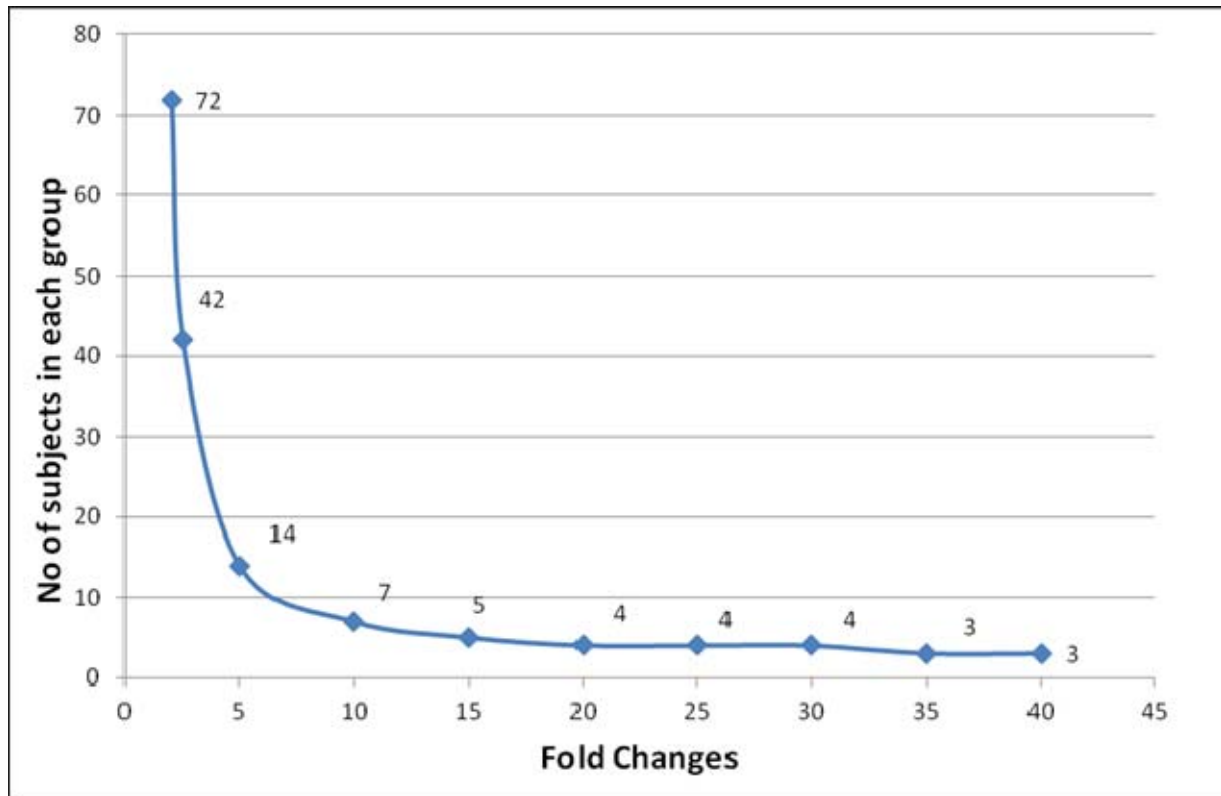


**Figure 3: Sample sizes for demonstrating a range of absolute difference in eosinophil percent between active and placebo group**

