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# Cumulative Enhancing Lesion Volume as Outcome Measure in MS Clinical Trials

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*Submitted for publication*

## ABSTRACT

**Background:** The number of enhancing lesions is an accepted measure for monitoring the effect of anti-inflammatory treatments in MS clinical trials. During treatment, enhancing lesions may still occur, but these lesions are significantly smaller. This effect may be missed by determining the number of lesions, but detected by using the volume of enhancing lesions, which is potentially a more sensitive and statistically powerful outcome measure.

**Objective:** To assess the distribution and statistical power of the cumulative volume of enhancing lesions compared to the number of enhancing lesions as primary outcome measure in MS clinical trials.

**Methods:** First, a literature search was performed to compare the effects of treatment on the number and the volume of enhancing lesions. Then, a mixture of the Binomial distribution and a chosen continuous distribution was proposed to describe the distribution of the volume of enhancing lesions in two dataset of RRMS patients monitored by MRI for 7 months and 9 months respectively. Next, based on the mixture-model, sample sizes for enhancing lesion volume as primary outcome measure were calculated.

**Results:** the effects between the volume and the number of enhancing lesions in literature were of comparable magnitude. The Weibull distribution proved the best fitting continuous model for describing enhancing lesion volumes in active patients, albeit the differences with the Gamma and Lognormal distribution were small. The power calculations showed approximately 94 patients to be required to detect a mean percentage decrease in enhancing lesion volume and percentage increase in inactive patients of 20%, assuming moderately active patients. For comparison, calculations for enhancing lesion counts showed approximately 129 patients are required to detect a 50% decrease in the mean *number* of lesions.

**Conclusion:** The mixture of the Binomial and Weibull distribution is a first approach in modelling new enhancing lesion volumes in MS, yielding feasible parametric sample sizes estimates for parallel grouped designed clinical trials, and shows an advantageous outcome measure compared to enhancing lesion counts in terms of study power.

## INTRODUCTION

Magnetic Resonance Imaging (MRI) is a sensitive tool to visualize the characteristic inflammatory activity in patients with Multiple Sclerosis (MS). On gadolinium (Gd) enhanced T1-weighted images, focal breaches of the blood brain barrier appear as contrast enhancing lesions and serve as an objective marker to monitor the extent of inflammation [1]. In MS clinical trials, the reduction of enhancing lesion number is often used as the outcome measure of choice while the reduction in volume of enhancing lesions is of secondary importance. Immunomodulatory compounds however, not only reduce the number of enhancing lesions, but also diminish the inflammatory activity and size of the lesions that still originate. Di Rezze *et al*. [2] showed that treatment with Interferon beta-1b (IFNB-1b) not only led to a decrease in the cumulative number of enhancing lesions, but also to a reduction of the size of the enhancing lesions originating during treatment. In a study by Gupta *et al*. [3] a comparable result was shown for re-enhancing lesions, where enhancing lesions appearing during treatment with IFNB-1b were significantly smaller than enhancing lesions arising during treatment with placebo. These studies show that total enhancing lesion volume accounts for two efficacy components (number and size) and thus, is a potentially more sensitive outcome measure to detect anti-inflammatory treatment effects compared to enhancing lesion counts alone. Moreover, use of enhancing lesion volumes is statistically advantageous being a continuous variable in contrast to lesion number, which is discrete and likely yields less statistical power.

With the widespread use of approved therapies altering the practice of clinical trials in MS, use of more sensitive and powerful outcome measures to maximize the ability of detecting treatment effects is becoming increasingly important. Therefore, the present study's objective is to assess the statistical power of the cumulative volume of enhancing lesions as primary outcome measure in MS clinical trials. First, a systematic literature search for treatment efficacies on enhancing lesions number and enhancing lesion volume is performed. Second, a statistical model for the distribution of enhancing lesion volume is proposed. Then, sample size calculations based on the range of reported treatment effects and the proposed statistical distribution are performed to estimate the sample sizes required for parallel grouped, placebo-controlled clinical trials, using the volume of enhancing lesions as primary outcome measure, and compared with the number of required patients for trials using the number of enhancing lesions.

## MATERIAL AND METHODS

### Literature search

The medline database was searched for MS clinical trials using Gd enhanced MRI as primary or secondary outcome measure. Based on titles and abstracts, full reports were selected and evaluated for inclusion. Trials of interest were those examining the efficacy of an immunomodulatory therapy in a placebo-controlled manner, using a serial monthly MRI protocol and reporting both the effect on the number of enhancing lesions as well as the volume of enhancing lesions.

### Patient cohorts

New enhancing lesion data from two datasets were at our disposal. Baseline demographics and characteristics are shown in Table 1. Dataset A (Oral Interferon beta-1a (IFNB-1a) study [4]) is a cohort of 169 RRMS patients who received varying doses of IFNB-1a or placebo orally every other day for seven months. The cohort is regarded as a natural history cohort as no clinical or MRI effect of any dose of IFNB-1a was observed. Patients were included when there were at least two clearly documented relapses within 24 months prior to study entry in conjunction with seven T2 lesions on the screening scan, or at least one clearly documented relapse within 24 months prior to study entry in conjunction with at least one Gd-enhancing lesion, and at least another three T2 lesions on the screening MRI. Dataset B (Oral Temsirolimus study [5]) is the placebo arm of a double-blind placebo-controlled multicentre trial. Patients were included if there was at least one documented relapse in the preceding 12 months before screening, or at least one documented relapse in the preceding 24 months before screening in conjunction with at least one Gd-enhancing lesion on the screening or baseline scan.

### MRI analysis

MRI data in dataset A and B were obtained from six and nine monthly follow-up scans respectively. All scans were performed in accordance with published guidelines for the use of MRI in clinical trials [6]. A radiologist marked all MS lesions on the original films, after which a trained technician manually outlined the lesions on each slice of the scan using purpose-developed software (Show-Images), using the markings as a reference. The volume of enhancing lesions, measured in milliliters, was determined by the sum of all lesion areas in a given scan, multiplied with the interslice distance.

**Table 1** | Baseline descriptives and MRI characteristics

| Characteristics | Dataset A (n=169) | Dataset B Placebo (n=69) |
|---|---|---|
| Patient | | |
| Female / Male | 123 / 46 | 50 / 19 |
| RRMS / SPMS with relapses | 169 / 0 | 57 / 12 |
| Mean age (SD) | 35.4 (8.4) | 38.5 (9.1) |
| Mean disease duration in years (SD) | 6.4 (5.3) | 5.9 (5.9) |
| Median baseline EDSS (IQR) | 2.0 (1.5 - 3.5) | 2.5 (1.5 - 3.5) |
| MRI | | |
| % inactive patients | 41.4% | 69.6% |
| Mean number of enhancing lesions (SD) | 2.4 (4.1) | 1.3 (0.4) |

RRMS=Relapsing Remitting Multiple Sclerosis
SPMS=Secondary Progressive Multiple Sclerosis
SPMS=Expanded Disability Status Scale

## Statistical methods

A) Statistical modelling of enhancing lesion volumes

The cumulative volume of enhancing lesions was considered as the primary outcome variable. A known characteristic of the distribution of lesion load data is its positive skewness, which typically is converted to a normal distribution by transformation of the original variable by, for example, a cubic root transformation [7]. With enhancing lesion volumes however, a problem arises due to the presence of a considerable amount of zero lesion volumes in patients who develop no enhancing lesions at all, which is not amenable by transformation. In the present study, enhancing lesion volume will be modelled using a two-component or mixture model. The first component determines the occurrence of inactive patients, and models the proportion of patients developing zero enhancing lesions over the study period by the Binomial distribution. The second component focuses on the cumulative enhancing lesion volume active patients develop during the study period. To determine the best fitting distribution for modelling the cumulative volume of enhancing lesions in active patients, a selection of six conceivable continuous distributions was fitted on the subset of active patients in both datasets: The Gamma, Weibull, Lognormal, Normal, Normal after cube root transformation, and Inverse Gaussian distribution. All models are basic and well known distributions, generally applicable in practice and characterized by two parameters [8]. The goodness of fit was assessed by means of the Anderson-Darling

and the Kolmogorov Smirnof test, with the best fitting model yielding the highest p-value (closest to 1).

B) Sample size calculations

The binomial mixture was implemented in a statistical resampling procedure using MATLAB™ (version 2007a), to simulate parallel-grouped, placebo controlled clinical trials. For the placebo group of each simulated trial, the proportion of inactive patients was randomly sampled from the binomial distribution with its parameter based on dataset A and, for each active patient, the cumulative volume of enhancing lesions was sampled from the chosen continuous distribution, again with its parameters derived from the population of dataset A. For the corresponding treatment group, the proportion of inactive patients is sampled from a binomial distribution, with the parameter $\mu_{treated} = \mu_{placebo}$ x (1 - treatment effect), and the cumulative volume of enhancing lesions is sampled from the chosen continuous distribution with the parameter $\mu_{treated} = \mu_{placebo}$ x (1-treatment effect). As such the treatment effect was expressed as the percentage difference in the mean proportion of inactive patients *or* the percentage difference in the mean cumulative enhancing lesion volume over the study period between a group of treated patients and a group of placebo patients. A total of 1000 trials were simulated for treatment effects ranging from 10% to 50% increase in the proportion of inactive patients, in combination with 10% to 50% decrease in the mean cumulative enhancing lesion volume of active patients, and statistical power was calculated as the proportion of trials yielding a significant result in either the percentage increase in inactive patients *or* the percentage decrease in mean enhancing lesion volume for a power of 80% (significance was determined by a Wilcoxon Rank sum test at a two-sided test level of 0.05).

For comparison, the number of required patients for trials using the number of enhancing lesions as primary outcome measure were calculated with a statistical re-sampling procedure based on a 1000 simulated trials, generated from the NB distribution with its parameters derived from the oral IFNB-1a study population [9]. Again, significance was determined by a Wilcoxon Rank sum test at a two-sided test level of 5%.

## RESULTS

### Literature search

A total of 29 MRI monitored clinical MS trials from 1999 to 2008 were identified as potentially relevant. 25 studies were excluded after examining the full published papers: 4 studies did not include enhanced T1 imaging, 16 studies did not assess or report enhancing lesion volumes, 1 study reported enhancing lesion volumes but no enhancing lesion counts, and 4 studies were not placebo-controlled. The results of the reports that met the specified inclusion criteria are shown in Table 2. Overall, the treatment efficacy measured by the percentage increase in inactive patients ranged from approximately 0% to 40%, and the reduction of cumulative enhancing lesion volume from 30% to 90% . However, when the effects on enhancing lesion volumes were recalculated for the active patients in isolation (thus patients truly responsible for the cumulative lesion volume), the effect range for enhancing volume reduction was approximately 10% to 60% reduction. We noted that the range of effects for the volume of enhancing lesions runs approximately parallel with the range of treatment effects for the number of enhancing lesions (overall mean effect of 56% and 58% reduction respectively).

### Statistical modelling

Table 3 shows the result of the Anderson-Darling and Kolmogorov Smirnof goodness of fit tests for the continuous distributions considered for describing the cumulative enhancing lesion volume of active patients (Easyfit 4.3, Mathwave Technologies). In both datasets, the Normal and IG distribution proved a poor fit for enhancing lesion volume data, whereas small differences were found between the remaining Weibull, Gamma, Lognormal and Normal (after cubic root transformation) distribution. Overall, the Weibull distribution performed best, and was elected to model the enhancing lesion volume of the active patients. Its fit is visualized in Figure 1.

### Sample size calculations

**Table 4** displays the results of the sample size calculations based on the cumulative enhancing lesion volume as primary outcome measure. A treatment effect on the Weibull distribution was defined according to alteration of the scale parameter, and the shape parameter was kept constant. This resulted in a constant coefficient of variation.

**Table 2** | Results of literature search. Effects shown are the % increase in active patients, the % change in mean or median number of lesions, the % change in mean lesion volume in the complete cohort, and the percentage change in mean lesion volume in active patients.

| Study | n* | fu-time (months) | % inactive patients placebo | Efficacy measure | | | |
|---|---|---|---|---|---|---|---|
| | | | | Absolute % increase in inactive patients | Lesion Number (all patients) | Lesion Volume (all patients) | Lesion Volume (active patients) |
| Oral Temsirolimus | 120 | 9 | 25% | +2% | -26% | -29% | -26% |
| Glatiramer Acetate | 239 | 9 | 5% | -2% | -33%** | -42% | -43% |
| Natalizumab | | | | | | | |
| 3 mg | 139 | 6 | 32% | +43% | -88% | -87% | -64% |
| 6 mg | 145 | 6 | 32% | +33% | -82% | -76% | -54% |
| Oral Fingolimod | | | | | | | |
| 1.25 mg | 164 | 6 | 47% | +30% | -43% | -50% | +16% |
| 5 mg | 158 | 6 | 47% | +35% | -61% | -63% | +10% |
| Overall mean effect | | | | 13% | 56% | 58% | 27% |

fu = follow up

* total number of participating patients in trial

** median lesion number

**Table 3** | Goodness of fit of conceivable continuous distributions. Results of the Anderson Darling and Kolmogorev Smirnof goodness of fit test for the cumulative volume of enhancing lesions in active patients in dataset A (n=133) and B (n=44). A higher p-value signifies a better fit.

| Distribution | Anderson Darling | | Kolmogorov Smirnof | |
|---|---|---|---|---|
| | Dataset A | Dataset B | Dataset A | Dataset B |
| Weibull | p>0.2 | p>0.2 | p=0.77 | p=0.77 |
| Gamma | p>0.2 | 0.1<p<0.2 | p=0.80 | p=0.12 |
| Lognormal | 0.1<p<0.2 | p>0.2 | p=0.06 | p=0.99 |
| Normal (cuberoot transformation) | p>0.2 | p>0.2 | p=0.60 | p=0.36 |
| Normal (ln transformation) | 0.1<p<0.2 | p>0.2 | p=0.06 | p=0.99 |
| Normal (Log10 transformation) | 0.1<p<0.2 | p>0.2 | p=0.06 | P=0.99 |
| Normal | p<0.01 | p<0.01 | p<0.01 | p<0.01 |
| Inv. Gaussian | p<0.01 | p<0.05 | p<0.01 | p=0.49 |

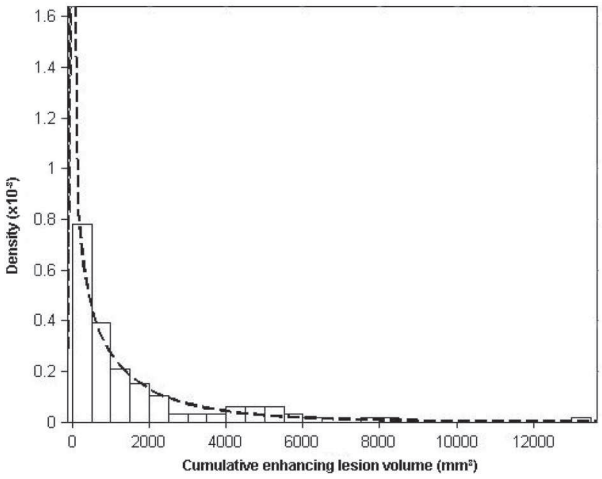


**Figure 1** | Visualization of the fit of the Weibull distribution on cumulative enhancing volume data of active patients in dataset A.

**Table 4** | Sample size estimates. Number of patients per treatment arm necessary to perform parallel group designed trials with a statistical power of 80%, to detect treatment effects ranging from 0% - 50% increase in inactive patients and a 0% - 50% reduction in mean enhancing lesion volume in active patients.

| % inactive patients in placebo group | % decrease in mean lesion volume of active patients | Absolute % increase in inactive patients | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0% | 10% | 20% | 30% | 40% | 50% |
| 10% | 0% | - | - | - | 435 | 225 | 124 |
| | 10% | 518 | 309 | 204 | 139 | 86 | 65 |
| | 20% | 128 | 101 | 82 | 65 | 47 | 38 |
| | 30% | 56 | 50 | 43 | 35 | 30 | 27 |
| | 40% | 31 | 28 | 24 | 22 | 21 | 19 |
| | 50% | 20 | 19 | 18 | 17 | 16 | |
| 40% | 0% | - | - | - | - | - | 580 |
| | 10% | 470 | 369 | 315 | 251 | 199 | 153 |
| | 20% | 121 | 115 | 94 | 83 | 75 | 66 |
| | 30% | 49 | v44 | 44 | 40 | 37 | 36 |
| | 40% | 25 | 24 | 23 | 24 | 21 | 20 |
| | 50% | 15 | 15 | 15 | 15 | 14 | 15 |

Shown are the required number of patients per treatment arm in a placebo-controlled clinical trial for the two effects responsible for changes in enhancing lesion volume: the percentage increase in inactive patients and the percentage decrease in mean volume in active patients.

When patients in the placebo group are assumed to be highly active (percentage of inactive patient 10%) approximately 82 patients are required to significantly detect a mean percentage decrease in enhancing lesion volume and percentage increase in inactive patients of 20%, whereas for the same treatment effects, 94 patients are required when patients in the placebo group are assumed to be moderately active (percentage of inactive patient 40%). In both scenarios, sample sizes decrease at a faster rate for increase in lesional volume effects than for the increase in effect on inactive patients.

For comparison, Table 5 displays the sample size estimates obtained for the *number* of enhancing lesions as primary outcome measure for placebo controlled clinical trials, obtained with the parametric re-sampling and simulation procedure based on the NB distribution [9]. With the NB-parameter estimates based on dataset A (mu=7.4, dispersion=0.45), the calculations show approximately 129 patients to

be required for detecting a 50% reduction in the mean number of enhancing lesions, fitting in with previous estimated numbers [10].

**Table 5** | Number of patients per treatment arm necessary to perform parallel grouped, placebo controlled clinical trials to detect a percentage decrease in mean number of enhancing lesions ranging from 30% to 90%, for a power of 80%.

| Effect size | n |
|---|---|
| 50% | 129 |
| 60% | 80 |
| 70% | 47 |
| 80% | 28 |
| 90% | 12 |

## DISCUSSION

The cumulative volume of enhancing lesions is a potentially attractive and conceivable alternative for measuring the amount of ongoing inflammation in patients with MS. To our knowledge, this is the first study addressing the statistical distribution of new enhancing lesion volumes and its statistical power as primary outcome measure in MS clinical trials. We found that the distribution of cumulative enhancing lesion volumes is adequately described by a mixture of the binomial distribution and the Weibull distribution, and found enhancing lesion volumes to be a potentially advantageous outcome measure compared to enhancing lesion counts in terms of study power.

When the estimated sample sizes are considered, it becomes apparent that a decrease in lesion volume in active patients has a more favourable effect on the required sample size compared to an increase in inactive patients, and that a more active cohort does not require more patients. Since both processes are likely to occur in parallel, these findings show that a decrease in measurable lesion volume lowers the detectability of treatment effects with a subsequent decrease in study power and confirms the expected advantage of selecting active patients for clinical trials.

Second, as the current analyses have shown, two parameters are necessary to adequately describe the distribution of new enhancing lesion volumes due to the presence of patients not developing new lesions during the follow-up period, and both parameters are affected when simulating treatment effects. The resulting sample

size estimates are reported in tabulated form to encompass both treatment effects. A formal comparison with sample size estimates for enhancing lesion counts therefore, becomes less "intuitive". Although the applied NB distribution also consists of two parameters, one of the parameters is assumed constant (parameter of dispersion), and a treatment effect was simulated by modifying the second parameter (parameter of location), in line with the methodology in previous studies [**9, 10**]. For simulations with enhancing lesion volumes however, this solution is unsuitable since a decrease in volume is always embedded in both parts of models coping with continuous data with an exact zero. Still, when the current sample size estimates for volumes and counts are considered, the order of magnitude of the sample sizes for enhancing lesion volumes is considerably lower than the estimates for enhancing lesion counts, even when there is no increase in inactive patients and treatment effects are solely driven by a decrease in enhancing volume of active patients (table 4, first column 0% increase in inactive patients).

This study is a first approach at statistical modelling enhancing lesion volumes in MS. Ultimately, it's parameterization would allow parametric analyses of treatment effects in clinical trials with subsequent estimation of treatment effects (instead of p-values) and adjustment for the effect of confounding variables in multivariate regression models. The chosen Weibull distribution is well known in statistical literature, and frequently applied for positive continuous data in numerous fields of research, *e.g.* survival analyses [8]. In theory, the Weibull distribution is applicable in the framework of a generalized linear model, with a logistic regression model describing the occurrence of an active or an inactive patient and the Weibull distribution modelling the enhancing lesion volumes in active patients. Although both datasets indicate the Weibull distribution being the optimal fit, the differences in fit with the Gamma distribution, the Lognormal distribution, and the Normal distribution on the cubic root transformed data are small. A definite choice for a model therefore, can only be validated when the fit is able to show consistent results in other datasets.

Mixture models are a common approach for handling data with excess zeros in various fields of research. One of the most well known distributions for modelling overdispersed data in this regard is the zero inflated Poisson (ZIP) distribution which models data by a mixture of a degenerate mass at zero and a Poisson distribution for the remaining values, but is applicable only for discrete data [11]. An alternative derivate approach are conditional or hurdle models, in which the presence or absence of data is modelled by an initial distribution, *e.g.* the binomial distribution, and

the remaining non-zero observations by a second distribution [12]. While the use of hurdle models for overdispersed discrete data is limited due to the availability of parsimonious alternatives such as the NB distribution [13], their generalizability to continuous distributions is a useful characteristic for data following a zero inflated poisson distribution with the nonzero observations deriving from a density function with strictly positive support [14]. Although currently the Weibull distribution proves the most promising distribution for describing enhancing lesion volumes, the present data also showed the Lognormal distribution not being substantially inferior to the Weibull distribution, and could prove a feasible alternative, as recently shown with the application of a zero-inflated log-normal model in human sperm cell DNA data, taking into account both inter- and intra-subject variations and the use of longitudinal data [15]. A disadvantage of the mixture approach is not being able to cope with the mixture of both discrete and continuous distributions concurrently. A promising approach in this regard is the Tweedie distribution, which has recently been found to model rainfall data and fishery catch processes [16,17]. When applied to enhancing lesion volumes, instead of considering lesional volumes as two separate circumstances (*e.g.* inactive patients and volumes in active patients) as proposed in this study, the Tweedie distribution models both processes concurrently in a single simplified model. In addition, the Tweedie distribution models processes by using generalized estimating equations, allowing adequate modelling of dependent and longitudinal data. Future analyses should prove whether this model adequately fits enhancing lesion volume data, and is practically applicable.

In conclusion, this study proposed a first approach in modelling new enhancing lesion volumes in MS by means of a mixture of the binomial and Weibull distribution. Parametric sample sizes estimates for parallel grouped designed clinical trials appeared feasible, albeit less intuitive to interpret. Alternative models should be explored in future studies.

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