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T2w Subtraction MR Imaging in Phase II MS Trials: A Powerful New Outcome Measure

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ABSTRACT

Objective: To compare long-interval T2-weighted subtraction (T2w-Sub) imaging with monthly gadolinium-enhanced T1-weighted (Gd-T1w) imaging for 1) detection of active lesions, 2) assessment of treatment efficacy, and 3) statistical power, in a multiple sclerosis (MS), phase II, clinical trial setting.

Methods: MRI data over 9 months from 116 patients (58 treatment, 58 placebo) from the oral temsirolimus trial were used. T2w-Sub images were scored for active lesions, independent of the original reading of the monthly Gd-T1w images. Treatment efficacy was evaluated using the nonparametric Mann-Whitney U test, and parametric negative binomial(NB)-regression, and power calculations were conducted.

Results: The mean number of T2w-Sub lesions in the treatment group was 3.0(\pm 4.6) vs. 5.9(\pm 8.8) for placebo; mean cumulative number of new Gd-T1w lesions in the treatment group was 5.5(\pm 9.1) vs. 9.1(\pm 17.2) for placebo. T2w-Sub imaging showed increased power to assess treatment efficacy compared with Gd-T1w imaging, when evaluated by: Mann-Whitney U test ($P=.017$ vs. $P=.177$), NB-regression without ($P=.011$ vs. $P=.092$) or with baseline adjustment ($P<.001$ vs. $P=.002$). Depending on the magnitude of the simulated treatment effect, sample size calculations showed reductions of 22%-34% in the number of patients (translating into reductions of 81%-83% in the number of MRI scans) needed to detect a significant treatment effect in favor of T2w subtraction imaging.

Conclusion: Compared with monthly Gd-enhanced T1w imaging, long-interval T2w subtraction MRI exhibited increased power to assess treatment efficacy, and could greatly increase the cost-effectiveness of phase II MS trials, by limiting the number of patients, contrast injections and MRI scans needed.

INTRODUCTION

Magnetic resonance imaging (MRI) is used as the primary outcome measure in multiple sclerosis (MS), phase II, clinical trials as it represents a more sensitive and reproducible method to monitor disease activity over time, compared with clinical measures [1,2]. Gadolinium(Gd)-enhancement is thought to reflect acute inflammation in which the blood-brain barrier is disrupted [3,4], persisting on average for around one month [5,6]. Anti-inflammatory properties of new treatments are thus assessed using serial Gd-enhanced T1-weighted (T1w) imaging, which is costly because of monthly scanning and contrast injections. Furthermore, the repeated administration of gadolinium could be potentially harmful, given the possible association between the use of Gd-based contrast media and the occurrence of nephrogenic systemic fibrosis, especially in patients with (chronic) renal insufficiency [7,8].

As the scanning interval between consecutive MRI scans is lengthened, an increasing amount of disease activity is likely to be missed when assessed solely by Gd-enhanced T1w imaging.

Therefore, trials of longer duration make supplementary use of T2-weighted (T2w) MR scans, performed at 3-monthly or even yearly intervals to monitor disease activity. In this setting, lesions on T2w images are thought to reflect the cumulative residual of disease activity [9]. However, the detection of active (new and enlarged) T2 lesions using serial conventional MR imaging is complicated, by repositioning errors and a background of unaltered non-active lesions [10].

T2w subtraction imaging, after image registration, provides an alternative in which the effect of repositioning errors is reduced, and the contrast between active lesions and the non-active background is enhanced as stable lesions cancel out. This method detected higher numbers of active lesions with greater interobserver agreement, compared with a standard analysis of nonregistered T2w images, in both a single and multi-center clinical trial setting [11,12].

Furthermore, subtraction imaging identified higher lesion volumes of disease activity, and demonstrated increased power to evaluate treatment efficacy using direct quantification of positive disease activity, compared with standard volumetric analyses [12,13]. Until now, longinterval T2w subtraction imaging has not been directly compared with serial Gd-enhanced T1w imaging for the identification of active lesions and assessment of treatment efficacy. If the residual disease activity identified by T2w subtraction imaging over many months exhibits at least the same power to evaluate

treatment efficacy compared with serial Gd-enhanced T1w imaging, this greatly would increase the cost-effectiveness of clinical trials (by limiting the number of patients, scans and contrast injections needed), and increase patient safety and compliance. Hence the goals in this study were to explore the applicability of long-interval T2w subtraction imaging to 1) detect active lesions, 2) assess treatment efficacy, and 3) secure statistical power, in comparison with serial monthly Gd-enhanced T1w imaging, in an MS, phase II, clinical trial setting.

MATERIALS AND METHODS

Patients and MR Image Acquisition

Serial MR images were derived from a multicenter, randomized, double-blind, placebo controlled, phase II, clinical trial, described in more detail in [14]. Briefly, 296 patients (with relapsing-remitting or secondary progressive MS with superimposed relapses) were randomized to 2, 4 or 8 mg temsirolimus orally every day (which selectively blocks interleukin-2 driven Tcell proliferation) or placebo for 9 months. Patients were not included in the trial if they had received systemic corticosteroid therapy within one month prior to randomization. In the present study only those patients randomized to either 8 mg temsirolimus (76 patients) or placebo (69 patients) were initially selected. Secondly, only patients with complete MRI data over all nine months were selected, which resulted in a final selection of 120 patients (61 patients on treatment and 59 on placebo). The clinical trial protocol was approved by local ethics review boards of all participating centers, and all subjects gave written informed consent.

Patients were monitored for nine months using monthly MRI scans, acquired according to published guidelines describing the use of MRI in clinical trials [15]. The MRI protocol included an oblique-axial, dual-echo, PD-weighted and T2-weighted, spin-echo or turbo/fast spin-echo (TR/TE of 2000-3000 / 15-40 & 60-100 ms) and a T1-weighted, spin-echo (TR/TE of 400-700 / 5-25 ms), before and after administration of 0.1 mmol/kg gadolinium diethylene triamine pentaacetic acid intravenously. Field of view for all examinations was 25 cm, combined with a 256 x 256 matrix, resulting in a roughly 1 x 1 mm pixel size in-plane. Images were acquired in two interleaved sets with a 3mm gap, resulting in whole brain coverage with contiguous 3mm thick slices.

Post-processing/registration

Subtraction images were generated by applying a series of post-processing steps. First, for both timepoints, a brain mask was created using the brain extraction tool (BET) [16], part of FMRIB's software library (FSL) [17]. All subsequent post-processing steps were calculated within the brain masks and subsequently applied to the whole scan, starting with an intra-scan intensity normalization (bias field correction) using the non-parametric method N3 [18], followed by a global scaling step to equate the average intensities of consecutive scans. Finally, images were spatially normalized (rigid body registration) to a halfway position using FMRIB's Linear Image Registration Tool (FLIRT) [19], with mutual information as cost function and sinc interpolation.

Image Analysis

The non-registered Gd-enhanced T1w images, had been centrally evaluated by the Image Analysis Centre (IAC), Amsterdam, The Netherlands, by expert reviewers blinded to treatment allocation. The number of new Gd-enhancing T1w lesions was assessed according to published guidelines [20] and available from the trial database. All subtraction images were evaluated by an expert reviewer (BM), also blinded to treatment allocation, using the freely available medical image processing, analysis, and visualization (MIPAV) software package developed at the National Institutes of Health, Bethesda, MD (<http://mipav.cit.nih.gov/>) [21]. The subtraction images comprising both the short and long echo T2w images were analyzed as one set. All subtraction images were analyzed alongside the halfway registered baseline and follow-up images, to ensure that changes identified on the subtraction images genuinely represented disease activity (rather than artifact). Lesions on subtraction images were identified according to published criteria which are described in more detail in [11,12]. Briefly, a lesion had to be a clearly visible, non-artifactual, area of change (bright or dark) against the grey background. In the subtraction image, new and enlarged lesions (positive activity) appear as bright areas, whereas resolved or shrunken lesions (negative activity) appear as dark areas. In the present study only new and enlarged lesions (positive activity) were identified, since these are used as outcome measure in MR monitored MS treatment trials and comparable to new activity detected by Gdenhanced T1w images.

Statistical Analysis

The correlation between the number of T2w subtraction lesions (month nine T2w scan minus the month one T2w scan) and the cumulative number of new Gd-enhancing T1w lesions (month two T1w scan through month nine T1w scan) was explored using Spearman's correlation coefficient. Lesion counts were expressed as means with accompanying standard deviations (SDs) for each imaging scheme, and compared using the Wilcoxon signed ranks test. By means of linear regression analysis, the predictive value of 1) the baseline number of Gd-enhancing T1w lesions, and 2) T2 lesion load (T2LL), for the development of on-study numbers of Gd-enhancing T1w lesions and T2w subtraction lesions, was determined. Statistically significant predictors were subsequently entered as covariates in the treatment efficacy analysis described below.

Treatment effects (reduction of active lesions) of oral temsirolimus as determined by the two imaging schemes was evaluated threefold; first, by means of the nonparametric Mann-Whitney U test; secondly, parametrically using a general linear model (GLM) based on the negative binomial (NB) distribution (NB-regression) *without* the inclusion of covariates; finally, again by means of NB-regression *with* the inclusion of covariates.

Subsequently, power calculations were conducted to estimate the required number of patients to determine a significant treatment effect. In addition, the number of MRI scans was calculated by multiplying the number of patients by two for T2w subtraction imaging, and eight for Gdenhanced T1w imaging. For the sample size estimates of T2w subtraction imaging the optimal fitting distribution for describing the number of T2w subtraction lesions was selected from a selection of conceivable statistical count distributions (Poisson, NB) by comparing their maximized log-likelihood values as measure of their goodness of fit of the data. Then, the statistical model was implemented using a parametric resampling and simulation methodology [22] which, for a given power, calculates the required sample sizes for a range of anticipated treatment effects (MATLAB version 2007a). For Gd-enhanced T1w imaging the same resampling and simulation procedure was applied, assuming the data to be NB distributed [23,24]. All statistical analyses were performed with SPSS version 15.0 (SPSS, Chicago, IL) and Stata version 10 (StataCorp, College Station, TX).

RESULTS

Lesion counts and correlations

In total 4(3.3%) of the 120 subtraction datasets were excluded from the visual analysis due to technical limitations; twice the registration procedure failed because the month one and nine T2w images were acquired with a different matrix size, and twice because extensive motion artifacts severely degraded the quality of the subtraction images. As a result, datasets from 116 patients were evaluated with both imaging schemes, of who the demographic descriptors are summarized in Table 1. The numbers of active (new and enlarged) T2w subtraction lesions and cumulative number of new Gd-enhancing T1w lesions detected were significantly correlated ($\rho = 0.80$, $P < .001$), albeit that the difference between both measures increased in patients with increasing lesion numbers (Figure 1). Overall, the mean cumulative number of new Gd-enhancing T1w lesions was significantly higher (1.7-fold) than the mean number of T2w subtraction lesions (7.28 vs. 4.41, $P < .001$, Figures 2-4).

Table 1 | Baseline descriptives

Characteristic	Placebo (n=58)	Treatment (n=58)
General		
No. Female (%)	43 (74)	38 (66)
No. RRMS* (%)	50 (86)	53 (91)
Mean age (SD)	38.3 (9.4)	38.6 (8.5)
Mean disease duration (SD)	4.7 (5.1)	5.1 (5.3)
Median baseline EDSS (IQR)	2.5 (1.5-3.5)	2.0 (1.5-3.5)
MRI		
No. ≥ 1 T1w Gd lesion (%)	19 (33)	35 (60)
Median T2 lesion volume in cm^3 (IQR)	4.8 (2.1-8.6)	4.6 (2.5-10.2)

IQR = Interquartile range, SD = standard deviation

* remaining patients have SPMS with relapses

The baseline T2LL significantly predicted the development of on-study T2w subtraction lesions ($\beta=2.464$, $P=.001$), and stratification by the median baseline T2LL showed a significantly (2.8-fold) higher mean number of lesions developing in the “above the median” group compared with the “below the median” group (6.5 vs. 2.3, $P < .001$). Likewise, the baseline number of Gdenhanced T1w lesions significantly

predicted the development of on-study Gd-enhanced T1w lesions ($\beta=2.189$, $P<.001$), and stratification by the median baseline number of Gd-enhancing T1w lesions also showed a significantly (3.6-fold) higher mean number of lesions developing in the “above the median” group compared with the “below the median” group (11.9 vs. 3.3, $P<.001$).

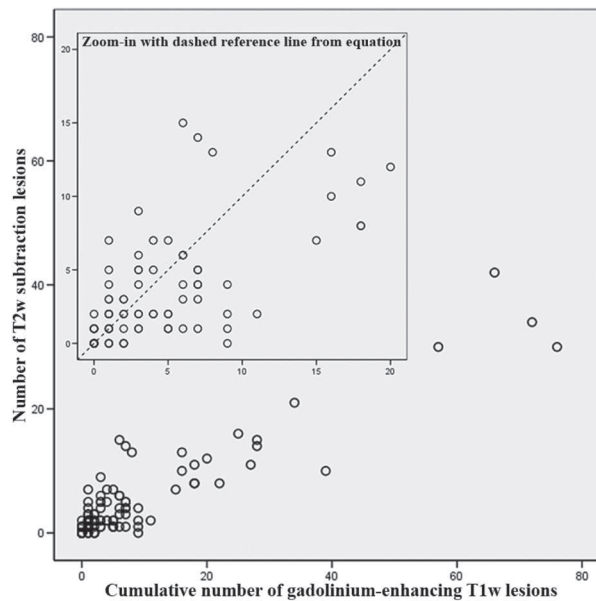


Figure 1. | Scatterplot visualizing the correlation (Spearman’s rho 0.80, $P<.001$) between the cumulative number of gadolinium-enhancing T1w lesions identified (horizontal axis) versus the number of T2w subtraction lesions identified (vertical) axis for all 116 patients.

Detection of treatment effect

The mean cumulative number of new Gd-enhancing T1w lesions identified in the treatment (temsirolimus) group was (38%) lower compared with the placebo group (5.5 vs. 9.1 respectively), but this difference was not significant when evaluated by using the Mann-Whitney U test ($P=.177$), nor by using NB-regression *without* covariates ($P=.092$, Table 2). The reduction in the cumulative number of new Gd-enhancing T1w lesions was deemed statistically significant only when either the baseline number of Gd-enhancing T1w lesions ($P=.005$), or baseline T2LL ($P=.011$), or both ($P=.002$), were entered as covariates in the NB-regression model.

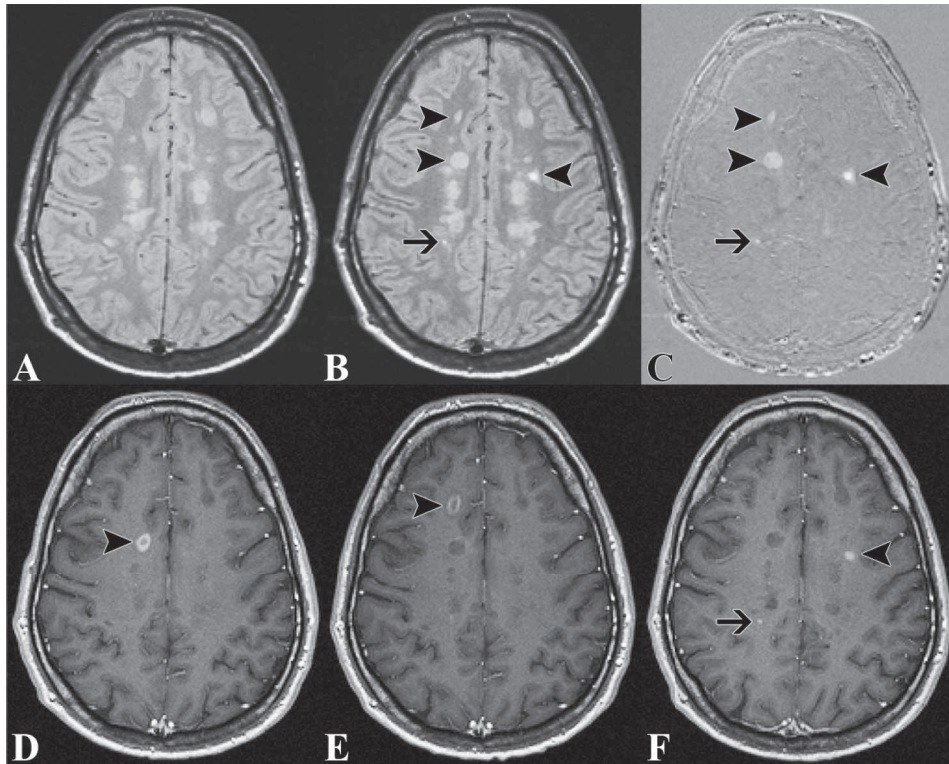


Figure 2 | Examples of Gd-enhancing T1w lesions visible as positive activity on the T2w subtraction image. MS activity on A, halfway registered month one T2w MR image; B, halfway-registered month nine T2w image; C, T2w subtraction image; D, E, F, month three, month five, and month nine non-registered Gd-enhanced T1w images respectively. Arrowheads = Gd-enhancing T1w lesions on various timepoints, also visible on the follow-up registered T2w image, and easily identified on the T2w subtraction image. Arrows = very small lesion not readily identified on the T2w subtraction image which did correspond to a Gd-enhancing T1w lesion.

The mean number of T2w subtraction lesions identified in the treatment group was (49%) lower compared with the placebo group (3.0 vs. 5.9, respectively). In contrast to the case using the number of Gd-enhancing lesions as outcome, this difference was significant with all the statistical methods used. In particular, the significance level was $P=.017$ when evaluated by using the Mann-Whitney U test, and $P=.011$ when using NB-regression *without* covariates (see Table 2 for more details).

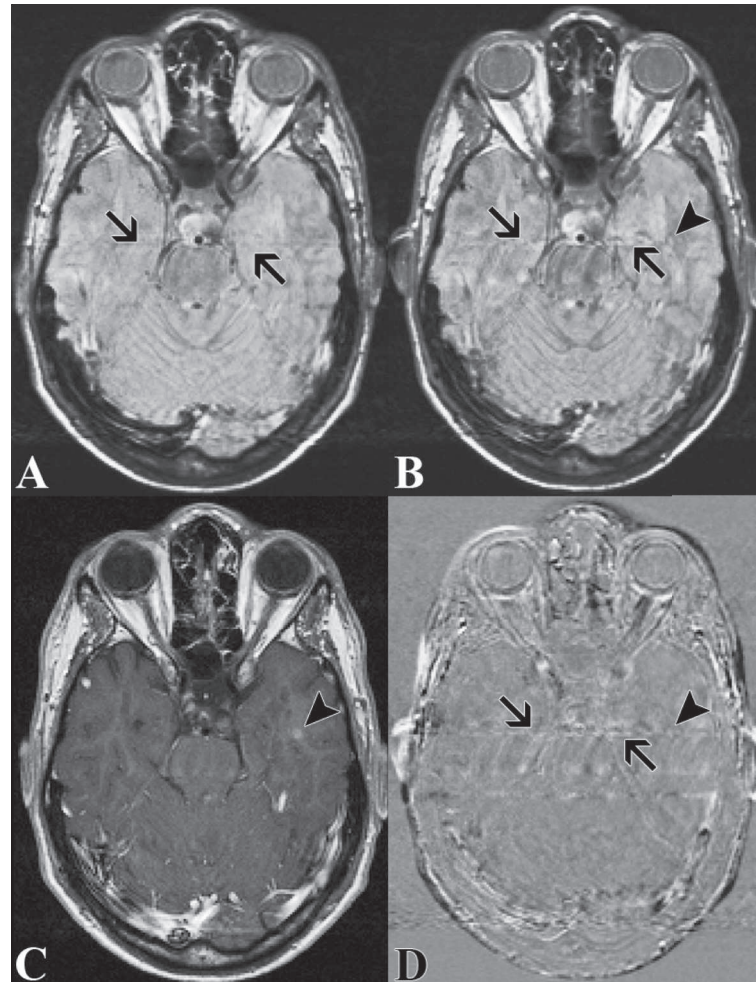


Figure 3 | Example of Gd-enhancing T1w lesion in the temporal lobe which was not identified on the T2w subtraction image. A, halfway registered month one T2w MR image; B, halfway-registered month nine T2w image; C, Gd-enhanced T1w image; D, T2w subtraction image. Arrowheads = Gd-enhancing T1w lesion on month two, not identified on the T2w subtraction image, due to flow artifacts (arrows) of the basilar artery.

Sample size calculations

Depending on the magnitude of the simulated treatment effect, the number of patients required per treatment arm to determine a significant treatment effect in a placebo controlled, parallel grouped clinical trial design, were 22 to 33 percent lower for T2w subtraction imaging, compared with Gd enhanced T1w imaging (Table

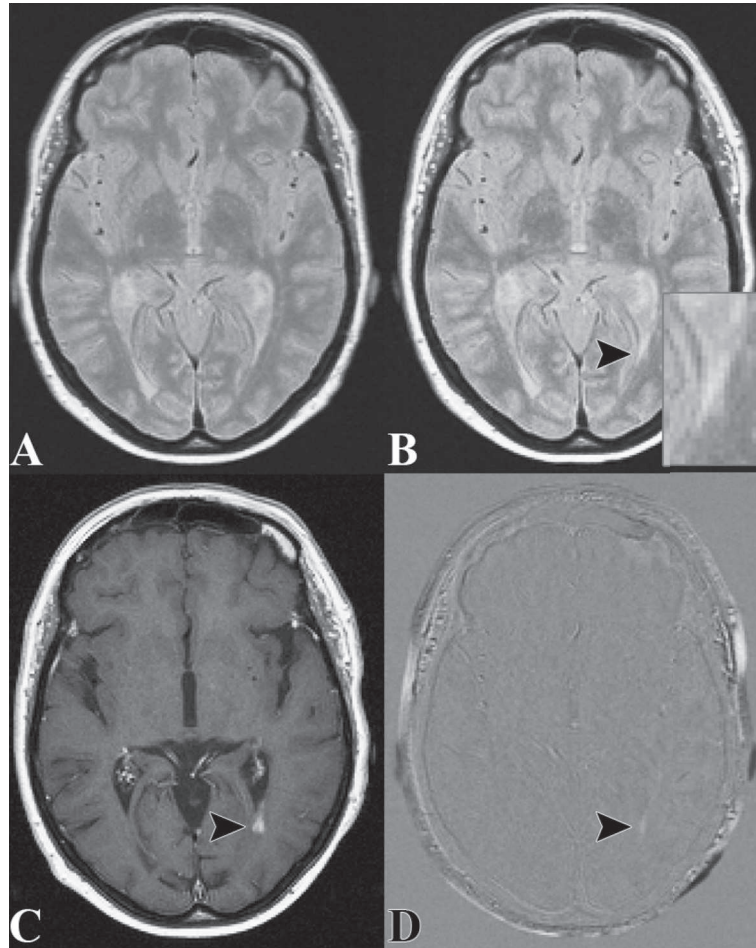


Figure 4 | Example of Gd-enhancing T1w lesion which was not identified on the T2w subtraction image. A, halfway registered month one T2w MR image; B, halfway-registered month nine T2w image; C, Gd-enhanced T1w image; D, T2w subtraction image. Arrowheads = Gd-enhancing T1w lesion on month two, seen as a very subtle lesion on the month nine registered T2w image, not identified on the T2w subtraction image.

3, Figure 5). This translated into reductions of 81% to 83% in the number of MRI scans needed to detect a significant treatment effect (Table 3). The point estimates are based on simulations with the following NB parameters derived from the data (T2w subtraction imaging: mean=5.9, dispersion parameter 0.58; Gd enhanced T1w imaging: mean=9.1 and dispersion parameter=0.41).

Table 2 | Analysis of treatment efficacy comparing patients on either temsirolimus or placebo, per analysis method

	Analysis method	
	T1w Gd	T2w Sub
Lesion Counts (Mean ± SD)		
Temsirolium (n=58)	5.5 ± 9.1	3.0 ± 4.6
Placebo (n=58)	9.1 ± 17.2	5.9 ± 8.8
	Analysis of treatment efficacy (P value)	
Mann-Whitney U test	.177	.017
NB-regression, adjusted for:		
no adjustment	.092	.011
no. of baseline T1w Gd lesions	.005	.002
baseline T2 lesion load	.011	<.001
both	.002	<.001

NB=negative binomial

Table 3 | Number of patients per treatment arm, necessary to perform parallel group designed trials with statistical powers of 80% or 90%, to significantly detect a mean percentage decrease from 30% to 90% in mean number of new lesions.

Treatment effect	80% Power			90% Power		
	T1w Gd	T2w Sub	% Reduction	T1w Gd	T2w Sub	% Reduction
30%	540	366	32	727	495	32
40%	266	184	31	371	245	34
50%	150	101	33	199	143	28
60%	88	60	32	120	82	32
70%	51	39	24	71	49	31
80%	30	22	27	44	29	34
90%	18	14	22	24	18	25

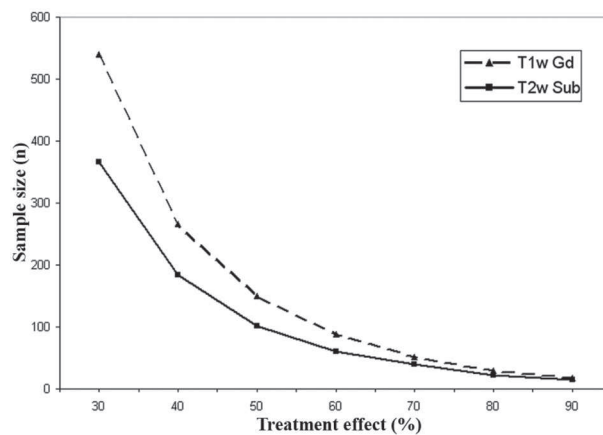


Figure 5 | Relationship between the number of patients needed in each treatment arm and various simulated treatment effects for a power of 80%, for both Gd-enhanced T1w imaging (dotted line) and T2w subtraction imaging (solid line).

DISCUSSION

A previous study has shown that T2w subtraction imaging detected higher numbers of active lesions with greater interobserver agreement and demonstrated increased power to evaluate treatment efficacy, compared with a standard analysis of non-registered (raw) T2w images [12]. In this study we found that despite an expected loss in sensitivity, long-interval T2w subtraction MR imaging also exhibited increased power to assess treatment efficacy, compared with monthly serial Gd-enhanced T1w imaging. In particular, the subtraction scheme used in our study detected around half of the lesions compared with Gd-enhanced T1w imaging. Several factors contributed to this finding. First, very small (1-2mm) lesions that were identified with Gd-enhanced T1w imaging were readily missed on subtraction images. The T2w images used in this study to create the subtraction images featured an anisotropic voxel size inducing subtle misregistration artifacts and blurring in the subtraction images, which particularly hampered the detection of (very) small lesions (Figure 2). This could be ameliorated by the application of 3D sequences with intrinsically higher signal-to-noise-ratios compared with 2D sequences [25-27], allowing the acquisition of smaller isotropic voxels, leading to a potentially more accurate registration result.

Secondly, flow artifacts, arising from the carotid and vertebrobasilar arteries and transverse sinuses, hampered the detection of lesions in the posterior fossa and temporal lobes on subtraction images (Figure 3). This could also be improved by the application of single-slab 3D sequences which employ non-spatially selective radio-frequency pulses [28,29], resulting in the absence of blood and cerebro-spinal fluid (CSF) flow artifacts, thereby improving the visualization of infratentorial lesions [27]. These two issues might be additional incentives to consider the implementation of single-slab 3D sequences in multicenter clinical trial design. Thirdly, some lesions only appeared as very subtle T2 hyperintensities on the month 9 T2w scan, and hence on the subtraction image, whereas their initial area of enhancement was quite extensive (Figure 4). This was most prominent for lesions identified with Gd-enhanced T1w imaging during the first months of the study, which had the largest time-window between the initial enhancement and the month nine T2w scan.

Conversely, some lesions were detected with T2w subtraction imaging that were not detected with Gd-enhanced T1w imaging (above the dashed reference line from equation in the zoom-in of Figure 1). Gd-enhanced T1w imaging was undertaken monthly in the oral temsirolimus trial. However, some new lesions, *i.e.* those new lesions that appear just after a monthly scan and enhance for only a couple of weeks, will be missed. Cotton et al reported a mean duration of enhancement of new lesions of 3.07 weeks [30], which makes it likely that some new lesions will not be captured with monthly Gd-enhanced imaging. Indeed, early literature already reported that not all new or enlarging T2 lesions demonstrate enhancement on monthly scanning [5,31,32]. A future study could conduct a direct side-by-side comparison of both imaging schemes per lesion, to determine the exact properties of Gd-T1w lesions (*e.g.* size, location, type of enhancement) that were not seen with T2w subtraction imaging, and vice versa the properties of T2w subtraction lesions that we not seen with Gd-T1w imaging.

Using the nonparametric Mann-Whitney U test we found a significant reduction (49%, $P=.017$) of the mean number of T2w subtraction lesions in the treatment (temsirolimus) group compared with the placebo group, whereas we found a non-significant reduction (40%, $P=.177$) of the mean cumulative number of new Gd-enhancing T1w lesions. Alternatively, we used the negative binomial (NB) distribution, which has been described to adequately model lesion count data over time [23,24], allowing a statistically more powerful parametric approach (*e.g.* general linear regression) to evaluate treatment efficacy (*i.e.* the reduction in active lesions) in

MS patients. Indeed, the aforementioned reductions in active lesions showed lower P values when evaluated by NB-regression compared with the non-parametric Mann-Whitney U test, which was more evident for Gd-enhanced T1w imaging (P=.092 vs. P=.177 respectively) than for T2w subtraction imaging (P=.011 vs. P=.017 respectively). Another advantage of using NB-regression is the possibility to adjust for (baseline) covariates, which can reduce the interpatient variance, and thereby increase the statistical power of MRI-monitored trials [22,33]. We also found this in our study, especially for Gd-enhanced T1w imaging, as NB-regression adjusted for the number of baseline Gd-enhancing T1w lesions and T2LL, resulted in a lower P value (P=.002), compared with unadjusted NB-regression (P=.092). Short of stratification for the aforementioned baseline determinants, statistical analysis of MRI-monitored phase II clinical trials should employ baseline covariate adjustment to increase statistical power.

Our study had several potential limitations. Firstly, the T2w MR images were acquired after the administration of gadolinium, resulting in a slightly increased signal intensity of Gd-enhancing lesions on T2w images [34]. Consequently, lesions that had a *higher* signal intensity on the month one scan than on the month nine scan (*i.e.* all lesions that showed Gd-enhancement on the month one scan), appeared as negative rather than positive activity and were not identified on the subtraction images. To make a rational comparison with Gd-enhanced T1w imaging, the cumulative number of new Gd-enhancing T1w lesions was calculated from the month 2 to the month 9 scans. Significance of all outcomes was maintained when including data from the month one Gd-enhanced T1w scans (data not shown). Secondly, 4 patients were excluded from our study due to technical limitations of the subtraction scheme. However, 25 patients (15 treatment, 10 placebo) included in the original trial, were excluded from our study because Gd data was not available for all nine months of follow-up, whereas the month one and month nine T2w scans were available. Thirdly, the analysis of T2w subtraction images was undertaken by one reviewer in a research setting, whereas the Gd-enhanced T1w images were reviewed by multiple reviewers, as part of the clinical trial, in accordance with published guidelines. A previous study has reported a very high ICC (expressing interobserver agreement) of 0.98 for the detection of positive disease activity on T2w subtraction images [12]. As such, the use of a single reader in this study has probably led only to a marginal overestimation of the value of T2w subtraction imaging. Also, the reductions in the number of MRI scans needed to detect a significant treatment effect comparing T2w subtraction

with serial monthly Gd-T1w imaging might be slightly lower for phase II trials with a shorter duration (e.g. 6 months). Finally, T2w subtraction imaging could have a lower specificity compared with Gd-enhanced T1w imaging for the detection of MS activity. Age-related white matter changes, appearing as T2 hyperintensities without enhancement, also termed leukoaraiosis, are frequently found on brain MRI in elderly populations and thought to be the resultant of small vessel disease [35,36]. These lesions could be mistakenly identified as new MS lesions on T2w subtraction imaging. However, the mean age of patients in this study was around 38 years which makes it highly unlikely that the T2w subtraction lesion identified were vascular in origin.

In conclusion, despite an expected loss in sensitivity, we found long-interval T2w subtraction MR imaging to exhibit increased power to assess treatment efficacy, compared with serial monthly Gd-enhanced T1w imaging, in an MS, phase II, clinical trial setting. T2w subtraction MR imaging could greatly increase the cost-effectiveness of phase 2 MS trials, by limiting the number of patients, contrast injections, and MRI scans needed, and decrease patients' risk by obviating gadolinium administration.

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