Permeability of the Blood–Brain Barrier Predicts No Evidence of Disease Activity at 2 Years after Natalizumab or Fingolimod Treatment in Relapsing–Remitting Multiple Sclerosis

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Objective: To investigate whether blood–brain barrier (BBB) permeability, as measured by dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI), can provide early detection of suboptimal treatment response in relapsing–remitting multiple sclerosis (RRMS).
Methods: Thirty-five RRMS patients starting on fingolimod or natalizumab, drugs with a common effect of decreasing lymphocyte influx into the central nervous system, were scanned with DCE-MRI at 3T prior to treatment and at 3 and 6 months posttreatment. We calculated the influx constant K_i, a measure of BBB permeability, using the Patlak model. Suboptimal treatment response was defined as loss of no evidence of disease activity (NEDA) status after 2 years of treatment.
Results: Subjects with loss of NEDA status at 2 years had a 51% higher mean K_i in normal-appearing white matter (NAWM) measured after 6 months of treatment, compared to subjects with maintained NEDA status (mean difference = 0.06ml/100g/min, 95% confidence interval [CI] = 0.02–0.09, $p = 0.002$). K_i in NAWM at 6 months was a good predictor of loss of NEDA status at 2 years (area under the curve = 0.84, 95% CI = 0.70–0.99, $p = 0.003$), and a value above 0.136ml/100/g/min yielded an odds ratio of 12.4 for suboptimal treatment response at 2 years, with a sensitivity of 73% and a specificity of 82%.
Interpretation: Our results suggest that BBB permeability as measured by DCE-MRI reliably predicts suboptimal treatment response and is a surrogate marker of the state of health of the BBB. We find a predictive threshold for disease activity, which is remarkably identical in clinically isolated syndrome as previously reported and established RRMS as investigated here.

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A large number of immunomodulatory disease-modifying therapies (DMTs) are now available for relapsing–remitting multiple sclerosis (RRMS). Their main objective is a reduction in the number and severity of relapses, occurrence of new or enlarging lesions on magnetic resonance imaging (MRI), and prevention or delay in the onset of secondary progressive disease. In European countries, natalizumab and fingolimod share the same indication as second-line therapies in highly active RRMS, or as first-line therapy for aggressive and rapidly evolving disease.[1] Natalizumab is a monoclonal antibody against the $\alpha 4\beta 1$ integrin receptor, which mediates lymphocyte adherence to

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the endothelium, thereby directly suppressing lymphocyte passage across the blood–brain barrier (BBB).[2] Fingolimod is an agonist of the sphingosine-1 receptor, inducing receptor internalization and thereby trapping encephalitogenic lymphocytes in lymph nodes,[3] preventing them from migrating into the central nervous system. Hence, although the mechanism of action of these two drugs is different, their final effect is the same, that is, a reduction of the absolute number of lymphocytes trafficking across the BBB, as demonstrated by an equivalent reduction in CD4 lymphocyte counts in the cerebrospinal fluid (CSF).[4] Both treatments have been shown to be highly efficacious in reducing relapse rates by 54 to 68%, reducing occurrence of new T2 lesions on MRI as well as the number of visibly contrast-enhancing lesions.[5–7] Despite the high overall efficacy, the treatment response is highly heterogeneous, and a subset of patients still experience disease activity.[8] To evaluate treatment response, the concept of no evidence of disease activity (NEDA), which uses a zero-tolerance threshold (no signs of disease activity in any of 3 domains) has been proposed as a treatment goal.[9–11] Evaluating NEDA status after 2 years of DMT may be a reasonable approach, because it holds a positive predictive value of 78.3% for no progression at 7 years, with only minor improvement for re-evaluation at years 3 to 5.[11] However, early detection of suboptimal treatment response is becoming increasingly important, both in the context of the increasing number of available therapies[5,12,13] and due to DMTs seeming to have their best effect in the early stages of disease,[14] but no current method or clinical variable exists that is able to perform such stratification.[8,15] We have previously reported that BBB permeability in multiple sclerosis (MS) normal-appearing white matter (NAWM), measured as the influx constant K_i by dynamic-contrast enhanced MRI (DCE-MRI), is abnormal when compared to controls, is a marker of recent clinical relapse activity, and is attenuated by disease-modifying treatment.[16] K_i in NAWM correlates with biomarkers of immune cell trafficking in the CSF, and predicts conversion from optic neuritis to MS 2 years after onset.[17] Hence, we hypothesize that K_i can stratify MS patients according to DMT response, here defined as loss of NEDA status at 1 and 2 years of second-line treatment. Furthermore, we aim to characterize the mechanistic relationship between K_i and cellular traffic, in the setting of treatments whose common end result is a reduction in lymphocyte traffic across the BBB.

Patients and Methods

Study Participants

We prospectively included all RRMS patients referred for MRI by the MS clinic at Rigshospitalet, Glostrup between August 2011 and November 2013 as part of an evaluation prior to initiation of natalizumab or fingolimod treatment. Inclusion criteria were: (1) an established diagnosis of MS, (2) clinical indication for treatment with either natalizumab or fingolimod, and (3) age = 18 to 59 years. Exclusion criteria were: (1) other concurring disease and (2) contraindication to MRI scan or MRI contrast agent. Eighty-five RRMS patients were assessed for eligibility, of whom 45 patients met the inclusion criteria and agreed to participate in a baseline scan (Fig 1). Thirty-five of these proceeded to initiation of either natalizumab or fingolimod, all of whom had a follow-up MRI performed at 3 months posttreatment. Twenty-nine patients participated in the 6-month posttreatment MRI. After study completion, 2 subjects were excluded, the first due to the occurrence of antinatalizumab antibodies, which resulted in treatment cessation after 5 months of treatment, and the second due to suspected side effects to fingolimod in the form of macular edema, which resulted in treatment cessation after 8 months. Follow-up MRI scans were performed as close as possible to the 3-month (mean = 97 days, standard deviation [SD] = 13 days) and 6-month time points (mean = 189 days, SD = 17 days) posttreatment using the therapy initiation day as reference and consisted of axial T2, axial fluid-attenuated inversion recovery (FLAIR), and axial postcontrast T1 of the cerebrum as well as DCE-MRI (see below for sequence parameters). Spinal cord assessment was performed at baseline, but not at the 3- and 6-month follow-up scans. We recorded any use of methylprednisolone during the course of the study, with the intention to postpone any 3- or 6-month follow-up scan by 2 months after completion of steroid treatment. Only 1 subject was treated with intravenous

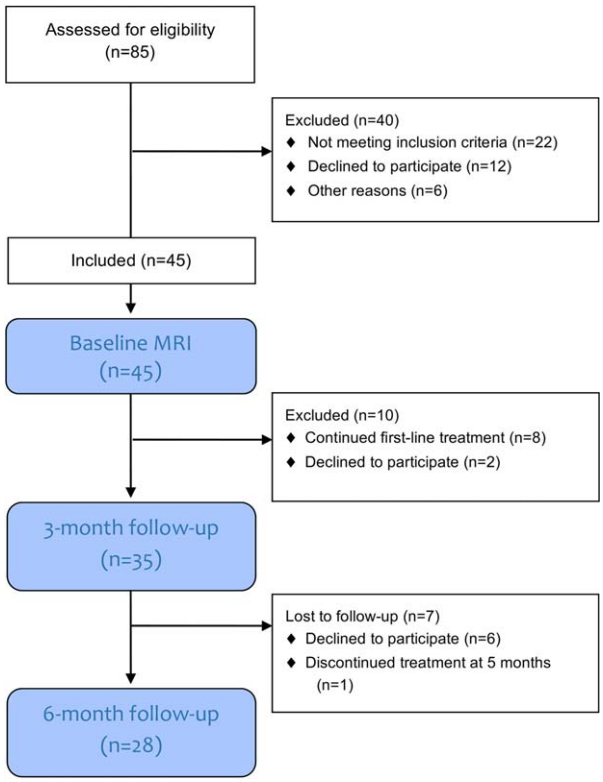

FIGURE 1: Subject inclusion procedure. MRI = magnetic resonance imaging. [Color figure can be viewed at www.annalsofneurology.org]

methylprednisolone during the first 6 months of the study. This occurred 4 months after treatment initiation, due to a major relapse 65 days before the planned 6-month scan, obviating the need for postponing the scan. Clinical data were obtained from hospital records 2 years after second-line treatment initiation for each individual subject. Collected variables were: baseline MS disease duration, baseline treatment status, history of methylprednisolone use, clinical relapses 12 months prior to second-line treatment initiation, and number of relapses, MRI activity, and Expanded Disability Status Scale (EDSS) at 1 and 2 years after treatment initiation. All subjects had regular clinical follow-up visits 3, 6, 12, 18, and 24 months post-treatment as part of standard clinical practice. Antinatalizumab antibodies were measured at 3, 6, 9, and 12 months. Collection of clinical data was performed by an experienced MS clinician who was blinded to the DCE-MRI results (another researcher analyzed the MRI data). Subjects who missed a clinical visit or an MRI were treated as missing data for the purpose of statistical analysis. Only subjects who completed a full 1 or 2 years of treatment were included in the 1- and 2-year analyses.

Outcome Measures

We used the following definitions. *Relapse* was defined as the appearance of new neurological symptoms or signs that lasted >24 hours in the absence of concurrent fever or illness.[18] The treating physician recorded relapses at the face-to-face visits at 3, 6, 12, 18, and 24 months. *Progression* was defined as an EDSS score increase of 1 or more points recorded at a biannual clinical visit that was sustained at the subsequent clinical visit 6 months later.[11,19] If the EDSS score was zero at baseline, progression was defined as an EDSS score change of 1.5 or more that was sustained at the subsequent clinical visit.[11] *MRI activity* was defined as new or enlarging T2 hyperintense lesions or T1 gadolinium-enhancing lesions in brain or spinal cord. To qualify as no evidence of MRI activity, new T2 hyperintense lesions and T1 gadolinium-enhancing lesions had to be absent on brain and spinal cord MRI. As recently suggested, disease activity occurring within the first 3 months after initiation of natalizumab or fingolimod treatment was disregarded when assessing NEDA status, to allow for development of a full treatment effect.[10,20] The earliest occurring loss of NEDA events within the 3 NEDA subdomains were (1) a new T_2 lesion at 6 months (this subject had another new T_2 lesion at 1 year thus also fulfilling loss of NEDA at 12 months), (2) a relapse at 7 months, and (3) an EDSS increase at 1 year. Thus, loss of NEDA status did not occur prior to the 6-month MRI scan.

Ethics

This study was approved by the Ethics Committee of Copenhagen County according to the standards of the National Committee on Health Research Ethics, protocol number H-D-2008-002. All experiments were conducted in accordance with the Helsinki Declaration of 1975, and all subjects gave written informed consent.

DCE-MRI

MRI was performed on a 3T magnetic resonance unit (Achieva; Philips, Best, the Netherlands) using a 32-element phased-array head coil. DCE-MRI used a T1-weighted saturation-recovery gradient-echo sequence with flip angle = 30°, repetition time = 3.9 milliseconds, echo time = 1.9 milliseconds, centric phase ordering, parallel imaging factor = 2, acquired matrix = 96×61, acquired voxel size = $2.40 \times 2.98 \times 8\text{mm}^3$ (interpolated to $0.90 \times 0.89 \times 8\text{mm}^3$), field of view = $230 \times 182\text{mm}^2$, 5 slices, slice thickness = 8mm. Data for an initial measurement of relaxation time (T1) and equilibrium magnetization (M0) were generated using a series of saturation time delays from 120 milliseconds to 10 seconds, covering the same slices as imaged during the bolus passage. The dynamic sequence used a saturation time delay of 120 milliseconds, giving a time resolution of 1.25 seconds, and 750 time points, corresponding to a total sampling duration of 15.7 minutes. The automatic bolus injection (Spectris; MedRad, Warrendale, PA) with speed 3ml/s followed by 20ml saline was started after the 10th time point. The dose of contrast agent (gadobutrol 1mmol/ml) was 0.045mmol/kg body weight. We acquired a separate slice at the level of the internal carotid artery to obtain an arterial input function with minimal partial volume for every subject. The remaining 4 DCE slices were used for defining regions of interest (ROIs) and subsequent estimation of tissue pharmacokinetic values. To achieve a full clinical dose of gadobutrol (0.1ml/kg), which is important for adequate detection of visibly contrast-enhancing lesions,[21] we injected the remaining contrast agent after the DCE acquisition and waited 5 minutes before acquiring the postcontrast T1 sequence.

MRI Sequences and ROIs

We used an axial T2-weighted MRI sequence (5 slices, echo time = 100 milliseconds, repetition time = 3,000 milliseconds, acquired voxel size = $0.57 \times 0.76 \times 8\text{mm}^3$ [interpolated to $0.45 \times 0.45 \times 8\text{mm}^3$], field of view = $230 \times 119\text{mm}^2$) with same orientation and slice thickness (8mm) as our DCE-MRI sequence, to manually draw ROIs in the periventricular NAWM, and in the normal-appearing thalamic gray matter in both hemispheres, avoiding inclusion of, or proximity to, any MS lesions or diffusely abnormal white matter, as previously described in detail.[16] Four ROIs were placed in periventricular NAWM (2 in the vicinity of the frontal ventricular horns [1 in each hemisphere] and 2 in the vicinity of the posterior horn [1 in each hemisphere]). Examples of ROI placement on anatomical images and corresponding K_i maps from 2 subjects can be seen in Figure 2. T2 lesions counts were performed by an experienced neuroradiologist using an axial T2 FLAIR sequence (35 slices, echo time = 125 milliseconds, repetition time = 11,000 milliseconds, acquired voxel size = $0.65 \times 0.99 \times 3.5\text{mm}^3$ [interpolated to $0.45 \times 0.45 \times 3.5\text{mm}^3$], field of view = $230 \times 119\text{mm}^2$, slice thickness = 3.5mm). ROIs were placed a minimum of 10mm from any MS lesion or CSF-containing structures. In the presence of contrast-enhancing lesions on a postcontrast axial T1-weighted spin echo sequence (44 slices, echo time = 10 milliseconds, repetition time = 600 milliseconds, acquired voxel size = $0.94 \times 1.25 \times 3\text{mm}^3$ [interpolated to $0.94 \times 0.94 \times 3\text{mm}^3$], field of view = $240 \times 240\text{mm}^2$, slice thickness = 3mm), we took care not to

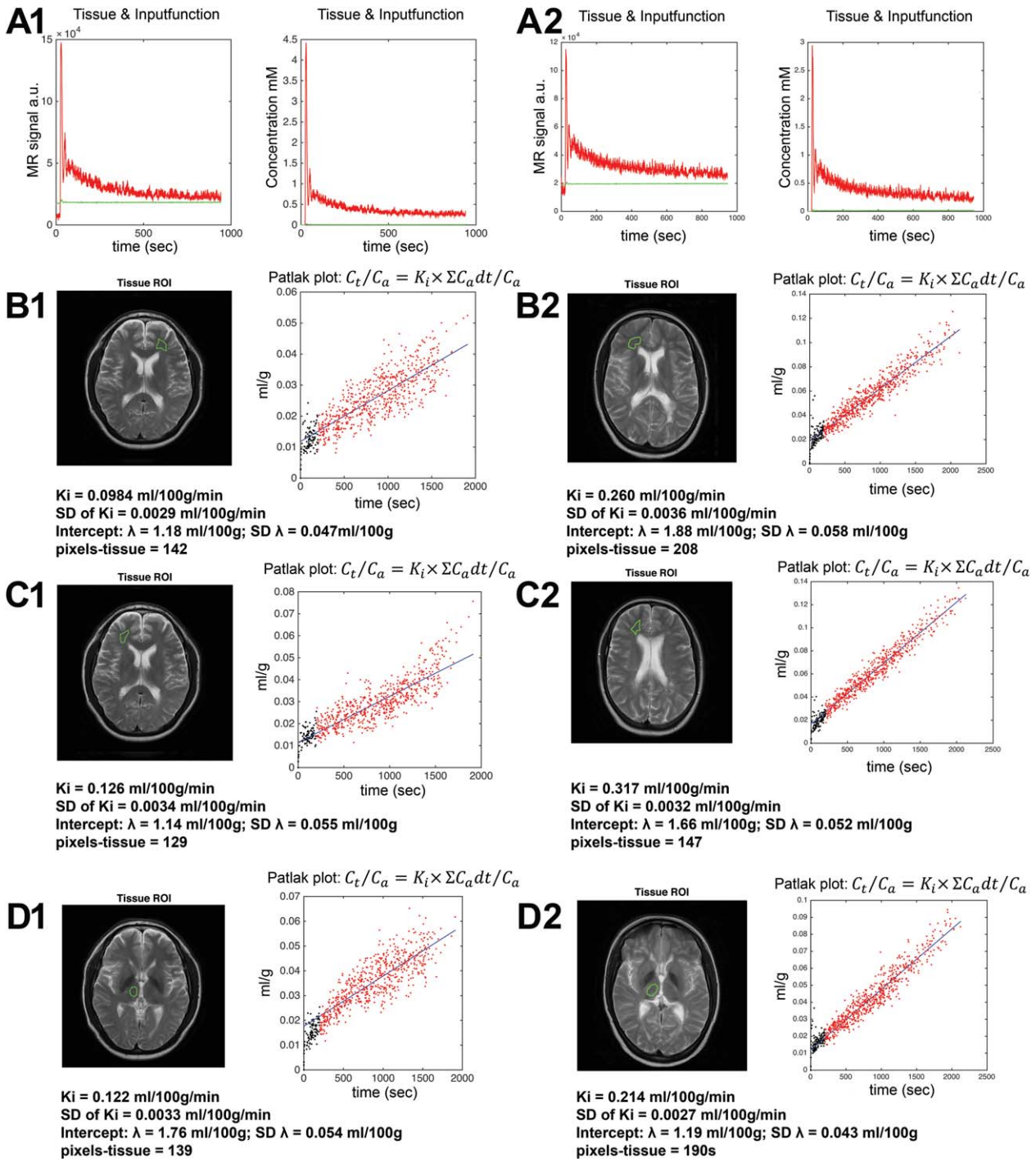

FIGURE 2: Arterial input function in arbitrary signal units (A, left) and concentration (A, right), and region of interest placement. Examples from 2 subjects are shown: left column from a subject with maintained no evidence of disease activity (NEDA) status after 2 years of treatment (A1, B1, C1, and D1) and right column from a subject with loss of NEDA status after 2 years of treatment (A2, B2, C2, and D2). Region of interest placement represents 2 examples in normal-appearing white matter (B, C) and 1 in thalamus (D) with their corresponding Patlak plots at 6 months posttreatment. a.u. = arbitrary units; MR = magnetic resonance; ROI = region of interest; SD = standard deviation. [Color figure can be viewed at www.annalsofneurology.org]

include the nearest 30mm of nonenhancing tissue. Our 4 DCE slices were placed with exactly the same angulation and anatomical position as the previous scan (evaluated for every scan). We ensured consistent positioning and size of our ROIs across different study time points by visual alignment with the previous scan.

Permeability Estimation

The DCE-MRI data were analyzed with a semiautomated procedure[22] using in-house MATLAB-based software. The DCE time series was converted to units of contrast agent concentration using T1 and M0, as determined from the multiple

saturation delay data, and a contrast agent relaxivity of $4s^{-1}/mM^{-1}$. The input function was measured in the voxel of the internal carotid artery with maximal signal change during the bolus passage and was corrected for partial volume by normalizing to a magnitude- and phase-derived venous outflow function, sampled in the sagittal sinus[23] ad modum Van Osch.[24] The median signal–time curve for all voxels in the ROI was extracted and used to calculate permeability. For each tissue type, we used the median value of permeability to exclude effects of possible outliers (eg, 4 regions of NAWM were drawn, of which the median was used to represent NAWM). Every subject was represented by 1 value calculated as a mean of the tissue-specific ROIs, as previously described.[16,17,25] Tissue concentration–time curves were evaluated using a combination of model-free deconvolution and a Patlak model, as described in previous work.[26] Permeability values, measured as K_i (full blood), relate to K^{trans} (plasma) by $K_i = K^{trans}/(1 - Hct)$. A fixed value of $Hct = 0.45$ was used throughout the study. Values of K_i are reported as ml/100g/min, assuming brain tissue density of 1g/ml.[27]

Statistics

Histograms, probability plots, and modified Kolmogorov–Smirnov (Lilliefors) testing were used to analyze continuous variables for standard normal distribution fit.[28,29] If the data were found to follow a normal distribution, 2-tailed Student *t* tests were used. If not, first a logarithmic transformation of the data was performed, and if normal distribution was not achieved, a Mann–Whitney *U* test was used. For comparisons between categorical data, chi-square tests were performed. We used a multiple linear regression approach to model the relationship between baseline K_i and MS clinical parameters. A 1-way repeated measures analysis of variance (ANOVA) was used to test for time effects after initiation of second-line treatment. Receiver operating characteristic (ROC) curves were used to estimate the predictive capability (area under the curve [AUC], and threshold with optimal sensitivity and specificity) of K_i to predict suboptimal treatment response, defined as loss of NEDA status. Logistic regression was performed to test for effects of multiple continuous independent variables on loss of NEDA status, and linear discriminant analysis was used when there were >2 possible outcomes. A p value < 0.05 allowed rejection of the null hypothesis. All analyses were performed in SPSS version 23 (IBM, Armonk, NY).

MULTIPLE COMPARISONS. The a priori hypothesis was that K_i after treatment initiation predicts suboptimal treatment effect, and we have thus investigated the performance of 4 different variables (K_i in NAWM and thalamus at 3 and 6 months). Applying a Bonferroni correction but taking the correlation coefficient (CC; average CC = 0.49) between the measured variables into account by way of the Dubey–Armitage-Parmar approach,[30,31] the threshold for rejecting the null hypothesis becomes $p = 0.024$. All p values are thus reported uncorrected, but only described as significant if falling below $p = 0.024$.

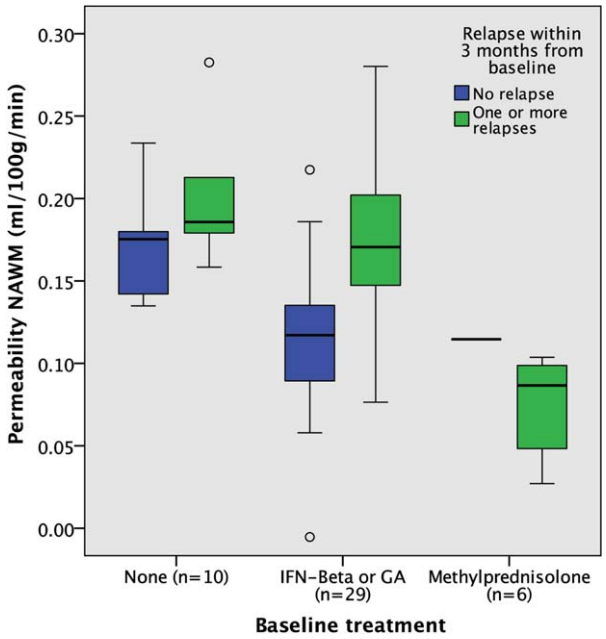

FIGURE 3: Baseline permeability in normal-appearing white matter (NAWM) for all subjects with a baseline scan (n = 45) according to current treatment and recent relapse. SPSS 23 standard setup for boxplot presentation was used. Black lines represent the median. Boxes represent the interquartile range (IQR; data between the 25% and 75% quartiles). Whiskers represent 1.5 times the IQR. Outliers *(open circles)* are defined as data points outside 1.5 × IQR. One subject was treated with pulsed steroids (50mg every first 3 days/month), and thus received methylprednisolone treatment despite not having had a recent relapse. GA = glatiramer acetate; IFN-Beta = interferon beta. [Color figure can be viewed at www.annalsofneurology.org]

Results

Baseline Data

Univariate linear regression analysis showed that baseline permeability in NAWM was predicted by methylprednisolone treatment 2 months prior ($\beta = -0.50$, $p = 0.003$), but not by days since last relapse ($p = 0.38$), first-line treatment (yes/no; $p = 0.53$), or visibly contrast-enhancing lesions (whether entered as yes/no [$p = 0.70$] or actual count [$p = 0.68$]). In multivariate analysis, methylprednisolone treatment 2 months prior ($\beta = -0.71$, $p = 0.00008$) and days since last relapse ($\beta = -0.48$, $p = 0.005$) predicted baseline K_i in NAWM (model $R^2 = 0.40$, $p = 0.0002$), but not first-line treatment (yes/no; $p = 0.55$) or visibly contrast-enhancing lesions ($p = 0.76$). In thalamus, baseline K_i was predicted by methylprednisolone treatment 2 months prior to baseline ($\beta = -0.45$, $p = 0.01$), but not by days since last relapse ($p = 0.58$) in univariate analysis. Baseline permeability according to current treatment and recent relapse can be seen in Figure 3.

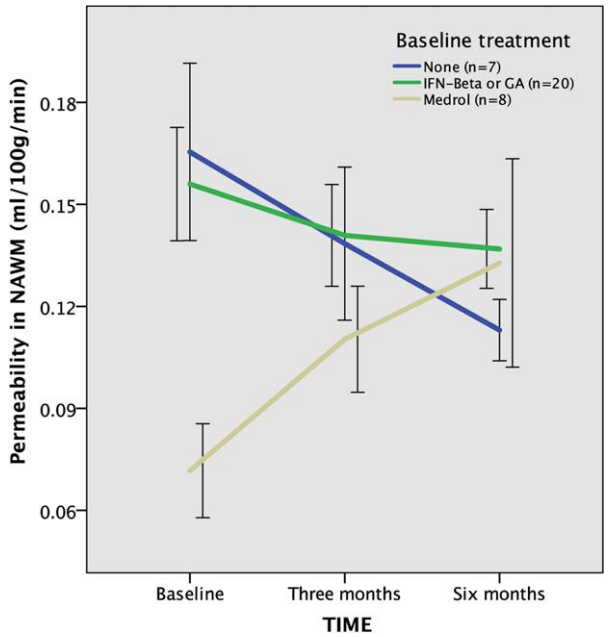

FIGURE 4: K_i in periventricular normal-appearing white matter (NAWM) during the course of second-line treatment for subjects who completed all 3 visits (n = 27). Bold lines represent mean K_i according to which treatment the subjects received prior to second-line treatment (solid gray = no prior treatment, black = interferon beta [IFN-Beta] or glatiramer acetate [GA], dashed gray = methylprednisolone within the past 2 months). Baseline scan was conducted shortly prior to second-line treatment initiation, and follow-up scans were conducted at 3 and 6 months after second-line treatment. Error bars represent ± 1 standard error of the mean. [Color figure can be viewed at www.annalsofneurology.org]

Treatment Effect

Between subjects receiving natalizumab and fingolimod there was no difference in mean K_i pretreatment (NAWM: mean difference = 0.008ml/100g/min, 95% confidence interval [CI] = −0.05 to 0.06, $p = 0.78$; thalamus: mean difference = 0.01ml/100g/min, 95% CI = −0.04 to 0.06; $p = 0.65$) and 6 months posttreatment (NAWM: mean difference = 0.004ml/100g/min, 95% CI = −0.04 to 0.05, $p = 0.84$; thalamus: mean difference = 0.0002ml/100g/min, 95% CI = −0.05 to 0.05, $p = 0.99$). However, at 3 months posttreatment, K_i in NAWM (mean difference = 0.06ml/100g/min, 95% CI = 0.02–0.10, $p = 0.002$) and thalamus (mean difference = 0.04ml/100g/min, 95% CI = 0.01–0.08, $p = 0.011$; both Student t tests) was higher in the natalizumab-treated patients, possibly reflecting a clinical selection bias favoring treatment of patients with highly active disease with natalizumab, as previously seen.[32,33] Of natalizumab-treated subjects, 3 of 11 had a relapse during the first 6 months of treatment (occurring 7, 8, and 133 days posttreatment) as opposed to 1 of 24 fingolimod-treated subjects (occurring 20 days posttreatment), possibly reflecting the same bias. A 1-way repeated measures ANOVA analysis with K_i in NAWM pretreatment and 6 months posttreatment as outcome and baseline methylprednisolone, days since last relapse, and first-line treatment as covariates found no significant effect of time ($p = 0.079$), but the interaction between time and baseline methylprednisolone showed a trend ($p = 0.041$; Fig 4). Significant between-subject covariates were baseline methylprednisolone treatment ($p = 0.001$) and days since last relapse ($p = 0.021$).

No Evidence of Disease Activity

After 1 year of second-line treatment, 12 of 35 subjects (34%) lost NEDA status. After 2 years, this increased to 15 of 35 (43%). Five of 11 (45%) natalizumab-treated subjects and 10 of 24 (42%) fingolimod-treated subjects had lost NEDA status at 2 years. Of the 15 subjects who lost NEDA status at 2 years, 4 subjects had activity in all 3 NEDA subdomains (relapse[s], new MRI activity, and EDDS increase), 3 subjects had relapse(s) and EDSS increase, 1 subject had new MRI activity and EDSS increase, 4 subjects had relapse(s) only, 2 subjects had EDSS increase only, and 1 subject had MRI activity only. Baseline demographics, clinical characteristics, and K_i values according to NEDA status at 2 years are shown in Table 1. Three subjects experienced a relapse shortly after starting treatment (7, 8, and 20 days after treatment initiation), but per protocol these were disregarded. Subjects who lost NEDA status at 2 years had a 51% higher K_i in NAWM at 6 months posttreatment (mean difference = 0.06 ml/100g/min, 95% CI = 0.02–0.09, $p = 0.002$) and a 78% higher annual relapse rate (ARR) 1 year pretreatment (mean difference = 0.93, 95% CI = 0.38–1.5, $p = 0.002$; all Student t tests), when compared to subjects who maintained NEDA status (see Table 1 and Fig 5). K_i at baseline and 3 months in NAWM and thalami were nonsignificant between NEDA groups (NAWM baseline: mean difference = 0.013ml/100g/min, 95% CI = −0.04 to 0.07, $p = 0.62$; thalamus baseline: mean difference = 0.02ml/100g/min, 95% CI = −0.03 to 0.07, $p = 0.39$; NAWM at 3 months: mean difference = 0.016ml/100g/min, 95% CI = −0.03 to 0.06, $p = 0.45$; thalamus at 3 months: mean difference = 0.02ml/100g/min, 95% CI = −0.02 to 0.06, $p = 0.25$); thalamic K_i at 6 months showed an insignificant trend for higher values (mean difference = 0.043ml/100g/min, 95% CI = 0.002–0.09, $p = 0.040$) in the loss of NEDA status group. In subjects who lost NEDA status at 1 year, only ARR 1 year pretreatment was significantly higher (mean difference = 0.99, 95% CI = 0.42–1.56, $p = 0.001$). An ROC curve with loss of NEDA at 2 years as outcome showed that K_i in NAWM at 6 months was a good predictor of loss of NEDA status at 2 years, with an AUC of 0.84 (95% CI = 0.70–0.99, $p = 0.003$; Fig 6). The optimal threshold, defined as the value that provided the highest

TABLE 1. Demographical, Clinical, and K_i Values according to NEDA Status 2 Years after Second-Line Treatment

Characteristic	NEDA Status at 2 Years		*p*
	Lost, n = 15	Maintained, n = 20	
Age, yr	36 (8.2)	43.1 (9.9)	0.03[a]
Female gender, n	9 (60%)	14 (70%)	0.72[b]
EDSS score at baseline	2.5 (1.6)	3.2 (1.4)	0.17[a]
Disease duration, yr	4.7 (3.7)	8.1 (6.8)	0.09[a]
Number of relapses 1 year before treatment start	2.1 (0.9)	1.2 (0.7)	0.002[a,c]
Last relapse onset, days	150 (124)	137 (110)	0.75[a]
Relapse within 3 months from baseline	8 (53%)	12 (60%)	0.74[b]
Baseline treatment			1.00[d]
None	3 (20%)	5 (25%)	
Interferon-β	10 (67%)	13 (65%)	
Glatiramer acetate	2 (13%)	2 (10%)	
Methylprednisolone < 2 months	4 (27%)	4 (20%)	0.70[b]
Days since treatment end[e]	27 (23)	39 (29)	0.80[a]
Baseline MRI			
T_2 lesion count	19.1 (12.7)	14.7 (8.5)	0.56[f]
T_2 lesion volume, mm^3	14.5 (15.2)	8.3 (3.5)	0.30[f]
$\geq$1 Gd^+ lesion	5 (33%)	5 (25%)	1.00[b]
Second-line treatment type = natalizumab	5 (33%)	6 (30%)	1.00[b]
K_i NAWM, ml/100g/min			
Baseline, n = 35	0.148 (0.078)	0.135 (0.072)	0.62[a]
Size, voxels	163 (92)	181 (102)	0.59[a]
3 months, n = 35	0.144 (0.049)	0.129 (0.062)	0.45[a]
Size, voxels	152 (92)	161 (83)	0.76[a]
6 months, n = 28	0.166 (0.059)	0.110 (0.029)	0.002[a,c]
Size, voxels	170 (99)	179 (86)	0.77[a]
K_i THAL, ml/100g/min			
Baseline, n = 35	0.152 (0.082)	0.131 (0.057)	0.39[a]
Size, voxels	129 (59)	110 (51)	0.31[a]
3 months, n = 35	0.143 (0.042)	0.125 (0.054)	0.25[a]
Size, voxels	115 (43)	130 (46)	0.33[a]
6 months, n = 28	0.165 (0.069)	0.122 (0.037)	0.04[a]
Size, voxels	143 (50)	136 (47)	0.67[a]

Values are mean ± standard deviation. K_i at 6 months and number of relapses before treatment start were significantly higher in subjects with loss of NEDA status at 2 years. K_i in thalamus at 6 months showed a trend for higher values but was nonsignificant.

[a]Student *t* test.

[b]Chi-square.

[c]Statistically significant.

[d]Chi-square with first-line treatment yes/no.

[e]Only entered for subjects who received steroid treatment within the past 2 months.

[f]Student *t* test on log-transformed data.

EDSS = Expanded Disability Status Scale; Gd^+ = Gadolinium enhancing lesion(s); MRI = magnetic resonance imaging; NAWM = normal-appearing white matter; NEDA = no evidence of disease activity; THAL = thalamus.

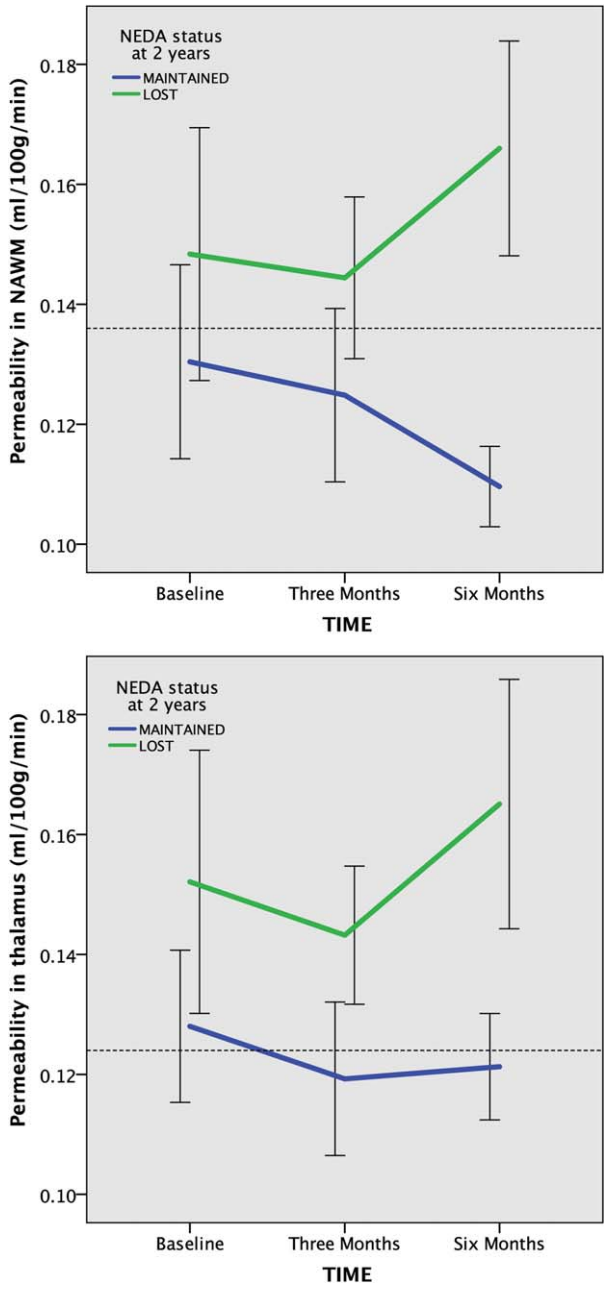

FIGURE 5: K_i in periventricular normal-appearing white matter (NAWM; top) and thalamus (bottom) before natalizumab or fingolimod treatment (baseline) and 3 and 6 months posttreatment. Horizontal dotted lines represent optimal threshold for loss of no evidence of disease activity (NEDA) status from the receiver operating characteristic curve analysis. Black line represents mean K_i in subjects with maintained NEDA status at 2 years, and gray line represents mean K_i in subjects with lost NEDA status. Error bars represent ± 1 standard error of the mean. [Color figure can be viewed at www.annalsofneurology.org]

added sensitivity and specificity[34] of K_i in NAWM for detecting loss of NEDA, was 0.136ml/100g/min, providing a sensitivity of 73% and specificity of 82%. More than 1 annual relapse 1 year pretreatment predicted loss of NEDA (AUC = 0.79, 95% CI = 0.64–0.94, $p = 0.004$) with a sensitivity of 87% and specificity of 65%. Univariate logistic regression analysis showed that K_i in NAWM at 6 months was associated with loss of NEDA at 2 years (an increase of 1 SD [0.05ml/100g/min] with an odds ratio [OR] = 10,4 95% CI = 1.4–74, $p = 0.02$), as was number of annual relapses 1 year pretreatment (OR = 9.2, 95% CI = 1.8–48, $p = 0.009$), but not presence of active T2 lesions at 6 months, K_i in NAWM at 3 months, K_i in thalamus at 3 or 6 months, age, gender, MS years, EDSS, baseline lesion count, or baseline contrast-enhancing lesions. Multivariate analysis with all the above-mentioned covariates showed that K_i in NAWM > 0.136ml/100g/min yielded an OR of 12.4 for loss of NEDA at 2 years, whereas > 1 annual relapse 1 year pretreatment was insignificant (Table 2). Two subjects switched to other therapies after 5 and 8 months, possibly influencing the NEDA outcome at 2 years. These were included in the primary analysis if they were on treatment while K_i was measured. Excluding these 2 subjects from the ROC curve analysis of K_i in NAWM at 6 months, with NEDA at 2 years as outcome, only caused minor changes to the results (AUC = 0.84, 95% CI = 0.70–0.99, $p = 0.003$, sensitivity 73%, specificity 81%).

DCE-MRI versus Conventional Contrast Imaging

Ten of 35 subjects (~29%) had 1 or more contrast-enhancing lesions on baseline MRI, and although these

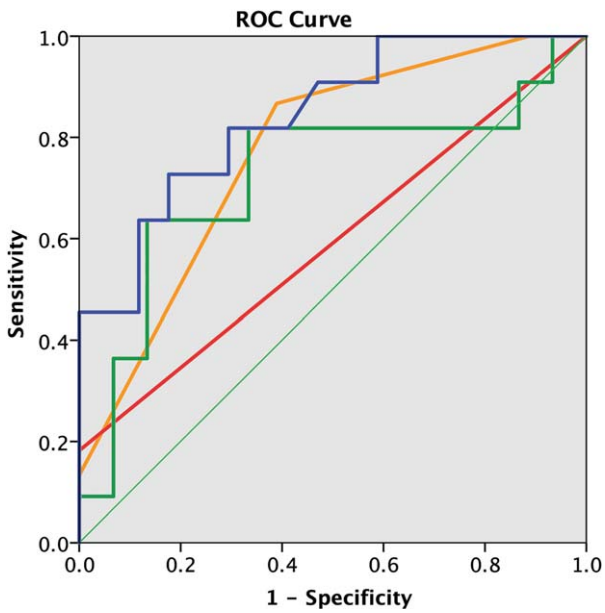

FIGURE 6: Result of receiver operator characteristic (ROC) curve analysis with loss of no evidence of disease activity status as outcome variable. Solid black line = K_i in normal-appearing white matter at 6 months; dashed black line = K_i in thalamus at 6 months; solid white line = annual relapse rate 1 year prior to treatment start; dashed white line = new active T2 lesions at 6 months. [Color figure can be viewed at www.annalsofneurology.org]

TABLE 2. Results of Stepwise Multivariate Logistic Regression with Loss of NEDA Status within the First 2 Years of Second-Line Treatment as Outcome Variable

Variable	Optimal Cutoff[a]	Predicted, n[b]	Observed, n (% correct)	*p*	Odds Ratio	95% CI
K_i in NAWM 6 months posttreatment	>0.136ml/100g/min	8 positives	11 (73%)	0.007	12.4	2 – 77
		14 negatives	17 (82%)			
K_i in the thalamus 6 months posttreatment	>0.124ml/100g/min	9 positives	11 (82%)	0.10	—	—
		11 negatives	17 (65%)			
Number of relapses 1 year before treatment start	>1	13 positives	15 (87%)	0.07	—	—
		13 negatives	20 (65%)			
Baseline T_2 lesion count	>13	10 positives	15 (67%)	0.94	—	—
		12 negatives	20 (60%)			
Active T_2 lesions at 6 months	>0	2 positives	11 (18%)	0.06	—	—
		18 negatives	18 (100%)			

Model Nagelkerke $R^2 = 0.37$, $p = 0.003$. K_i in NAWM and thalamus are significant predictors of loss of NEDA status at 2 years. Number of relapses 1 year before treatment start showed a trend but was nonsignificant.

[a]From receiver operating characteristic curve analysis.

[b]Predicted loss of NEDA status.

CI = confidence interval; NAWM = normal-appearing white matter; NEDA = no evidence of disease activity.

subjects had higher mean values of K_i at baseline (NAWM, 0.15ml/100g/min; thalami, 0.15ml/100g/min), the difference was not significant when compared to subjects without contrast-enhancing lesions (NAWM, 0.14ml/100g/min; thalami, 0.14 ml/100g/min; mean difference: NAWM, 0.01ml/100g/min, 95% CI = –0.05 to 0.07; thalamus, 0.01ml/100g/min, 95% CI = −0.03 to 0.05). Only 2 subjects had contrast-enhancing lesions on the 3-month follow-up MRI, and no subjects showed contrast enhancement at the 6-month follow-up. We found no correlation between gadolinium-enhancing lesions at baseline and permeability at baseline, 3 months, or 6 months.

Discussion

Mechanistic Insights

This study enables investigation of the mechanistic relationship between K_i (as measured by DCE-MRI) and cellular trafficking, by manipulation with disease-modifying treatments, which decrease cellular traffic across the BBB. We have previously found a correlation between K_i and cellular traffic into the CSF, and absence of correlation between K_i and albumin quotient, perhaps suggesting that K_i may be a surrogate marker of cellular influx.[17] This study addresses this issue, because the subjects maintaining NEDA represent an experimental situation where cellular traffic has been inhibited pharmacologically. We observe an apparent delay in the effect on K_i such that 6-month but not 3-month K_i predicted NEDA after 2 years. This lag is suggestive of an indirect process as opposed to an immediate effect of decreased cellular influx on K_i—which could reflect healing of the BBB solute barrier in the first 3 months after initiation of treatment. In those losing NEDA, one can hypothesize ongoing damage to the BBB. Hence, the association between cellular traffic and K_i may not be direct, but more likely represents a sequence of events where changes in cellular influx predate changes in the physical integrity of the BBB solute barrier. Hence, solute BBB permeability appears to be a prognostic marker, by reflecting the "state of health" of the BBB. This study illustrates, in vivo, the fundamental difference between cellular and solute traffic in man. Dissociation between cellular traffic and solute permeability has been observed in vitro, where interferon beta reduces lymphocyte transmigration, while having no effect on the permeability to albumin.[35]

We have previously reported that a $K_i > 0.13$ml/100g/min identifies optic neuritis subjects with high risk of conversion to RRMS, adding significant value

compared to using T2 lesions alone.[17] It is very interesting to note that the ROC threshold for further disease activity is identical in clinically isolated syndrome[17] versus established RRMS. This solute BBB permeability threshold could act as a reproducible surrogate marker for a BBB state associated with active disease.

Prediction of 2-Year NEDA Status

We also report the novel finding that a single measurement of BBB permeability in NAWM performed 6 months after initiation of natalizumab or fingolimod is capable of predicting loss of NEDA status within the first 2 years of treatment. Because NEDA at 2 years has recently been shown to predict disability progression as measured by EDSS at 7 years nearly as well as NEDA at 5 years,[11] measurements of BBB permeability could provide pivotal clinical information on treatment effect in the individual patient and possibly even provide long-term prognostic information. To our knowledge, no current method is capable of comparable stratification of treatment response to natalizumab or fingolimod. We find that K_i in NAWM > 0.136ml/100g/min predicts loss of NEDA at 2 years (OR = 12.4). For comparison, ≥ 2 contrast-enhancing lesions in the first year of treatment with interferon beta-1a identifies people at high risk of disability progression 15 years later. The OR for this effect was 8.9, one of the highest reported in the MS prediction literature.[36]

Presence of visibly contrast-enhancing lesions was not a significant determinant of K_i in NAWM or thalamus at baseline, 3 months, or 6 months. Furthermore, K_i in NAWM and thalamus was highly correlated in the same subjects at baseline (Spearman CC = 0.86), 3 months (CC = 0.88), and 6 months (CC = 0.82). Thus, the predictive effect of K_i is unlikely to be a carryover from the prognostic effect of contrast-enhancing lesions or a result of spillover of contrast agent from enhancing lesions into the surrounding NAWM. Assuming a textbook value of water diffusion in brain tissue of $1.0 \times 10^{-9} m^2/s$,[37] the distance a water molecule that has been in contact with contrast agent can diffuse during our DCE acquisition of 15 minutes is $10^{-9} m^2/15 \times 60 = 0.95$mm. Thus, it is highly unlikely that water from a gadolinium-enhancing lesion would diffuse into our NAWM ROIs. However, one study found contrast-enhancing lesions in the first year of natalizumab treatment to predict future disease progression.[38] In our cohort, contrast-enhancing lesions were only seen in 2 subjects at 3 months and none was observed at 6 months or at 1 year. Despite this, K_i at 6 months predicted NEDA at 2 years. This highlights the value of DCE-MRI in the detection of diffuse low-level BBB leakage, as distinct from the focal high-level leakage detected by conventional contrast MRI, most likely reflecting the different pathological processes in MS lesions and NAWM. The acute MS lesion is characterized by demyelination, axonal damage, gliosis, lymphocyte and macrophage infiltrates, and focal BBB damage, whereas NAWM, despite retaining myelin, often exhibits axonal swelling, activated major histocompatibility complex II^+ microglia and macrophages, gliosis, increased expression of proteolytic enzymes, and diffuse vessel leakage.[39–41] DCE-MRI has the additional advantage of using a lower dose of gadolinium contrast compared to conventional contrast MRI.

Subjects with loss of NEDA at 2 years had significantly more relapses in the year preceding treatment initiation. However, baseline ARR and lesion count are not significant in the regression analysis of K_i on NEDA; there is no correlation between 6-month K_i and days since last relapse or baseline contrast-enhancing lesion count; we only observed 1 relapse in close proximity to the 6-month scan. This dispels the possibility that the predictive effect of the higher K_i at 6 months on 2-year NEDA is a throwback to higher baseline disease activity.

Possible Reasons for Treatment Failure

We found that $K_i > 0.136$ml/100g/min predicts suboptimal natalizumab or fingolimod treatment response with a sensitivity of 73% and specificity of 81%. Possible reasons for treatment failure include: (1) lack of compliance, (2) neutralizing antibodies, (3) uncoupling of the disease process from the drug's mechanism of action, and (4) high intrinsic disease activity. Lack of compliance and neutralizing antibodies were excluded in this study, because patients were monitored for both. Uncoupling of disease and drug mechanism of action may occur if the inflammatory process is self-driven within the brain or alternative pathways have developed that allow persistent encephalitogenic leucocyte entry into the brain, for instance, higher expression of human leucocyte antigen I, chemokines, and selectin ligands at the BBB and/or structurally damaged endothelium. When treatment failure is due to high intrinsic disease activity, the drug is effective at its target but the individual's disease is so active that the therapeutic effect is not enough and disease activity breaks through.

K_i Response

In our general linear model, we find a trend toward an interaction effect of time and baseline methylprednisolone treatment, indicating a treatment effect between paired baseline and 6-month K_i only if baseline methylprednisolone is accounted for. This is indicative of a cumulative "K_i response" to both types of treatment, that is, a decrease in K_i at baseline due to methylprednisolone

and a decrease in K_i during the course of treatment with fingolimod or natalizumab. Taken together, this indicates that K_i response may provide a measure of treatment response. To further elucidate this relationship, one would need to follow individual patients over the course of several treatment regimes, encompassing both suboptimal and optimal treatment responses in the same individuals.

Proportion of NEDA

In this study, the proportion of subjects with loss of NEDA status was 34% during the first year and 43% during the second year. One natalizumab study reported loss of NEDA status at 2 years in 38% of subjects, but that study used less stringent MRI criteria not including enlarging T2 lesions.[42] In the AFFIRM trial, 63% had lost NEDA status after 2 years of natalizumab treatment, using the same NEDA criteria as in our study.[19] A head-to-head comparison of NEDA in natalizumab versus fingolimod treatment showed loss of NEDA status at 2 years in 30% and 77%, respectively.[43] The large discrepancy in the proportion of subjects with loss of NEDA status could likely represent differences in patient selection. In clinical trials, subjects with highly active disease are often favored for inclusion, to show maximum effect of the treatment, whereas in this study we included all patients starting on natalizumab or fingolimod treatment during the given time window.

Permeability Changes in the Context of Natalizumab or Fingolimod Treatment

Soon et al investigated T1-weighted signal intensity changes after gadolinium–diethylenetriamine penta-acetic acid administration in 27 RRMS patients after 24 weeks on natalizumab treatment but found no effect of treatment on signal change in NAWM when compared to 13 patients receiving placebo.[44] No clinical parameters, such as recent methylprednisolone treatment, relapses, and individual treatment effect, were taken into account; these are variables that we have shown govern K_i. This emphasizes the importance of including clinical covariates when characterizing changes in K_i over time.

Solute permeability across the BBB, which is mainly governed by diffusion,[45,46] is not synonymous with T-cell migration across the BBB, which is a highly regulated receptor-mediated process. To assess BBB permeability in this study, we used a macrocyclic gadolinium chelate (gadobutrol; Gd-BT-DO3A), which is a 547Da highly hydrophilic molecule.[47] Thus, even though natalizumab blockage of the VLA-4 receptor results in reduced migration of T-cells across the BBB, this does not necessarily imply a change in solute permeability per se.[45,46,48]

The time delay observed in this study of the effect of treatment on K_i, and the lack of correlation between baseline visibly contrast-enhancing lesions and K_i at any time point, indicate that solute permeability in NAWM is secondarily modulated by a treatment-related reduction of low-grade inflammatory activity. In summary, we find that a single DCE-MRI at 6 months after initiation of natalizumab or fingolimod treatment provides information on the state of health of the BBB that enables reliable stratification of treatment response. Thus, DCE-MRI can enable early detection of long-term suboptimal treatment response in RRMS and a personalized medicine approach to treatment, a limitation being the long scan time (15 minutes). These results and the proposed thresholds require validation in larger studies.

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Author Contributions

Study concept and design: S.P.C., J.L.F., H.B.W.L. Data acquisition and analysis: S.P.C., H.J.S., J.L.F., H.B.W.L. Drafting the text and figures: all authors.

Potential Conflicts of Interest

S.P.C. has received research funding and travel funding from Biogen Idec. J.L.F. has served on scientific advisory boards for and received funding for travel related to these activities as well as speaker honoraria from Biogen Idec. H.B.W.L. has received research funding from Biogen Idec. Biogen Idec produces and benefit from sales of natalizumab, which was investigated in the present study. However, Biogen Idec had no influence on study setup, subject inclusion, data analysis, interpretation of results, or publishing decisions, and intellectual rights belong to the authors alone. H.J.S., I.G., and A.V. report no conflicts of interest for the present study.

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