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Blood-brain barrier permeability changes in the first year after alemtuzumab treatment predict 2-year outcomes in relapsing-remitting multiple sclerosis

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ABSTRACT

Background: In relapsing-remitting multiple sclerosis (RRMS), early disease control reduces the risk of permanent disability. The blood–brain barrier (BBB) is compromised in MS, and its permeability is a potential biomarker.
Objective: To investigate BBB permeability measured by MRI as a marker of alemtuzumab efficacy.
Methods: Patients with RRMS initiating alemtuzumab treatment were recruited prospectively. BBB permeability was assessed as the Patlak-derived influx constant (K_i) by dynamic contrast-enhanced MRI before and 6, 12, and 18 months after the first course of alemtuzumab. No Evidence of Disease Activity-3 (NEDA-3) status was ascertained two years after treatment initiation.
Results: Patients who maintained NEDA-3 status at two years ($n = 7$) had a larger decrease in K_i between baseline and six months (-0.029 ml/100 g/min [CI -0.005 – -0.053]) and between baseline and 12 months in normal appearing white matter (0.043 [CI 0.022 – -0.065]), than those who experienced disease activity ($n = 8$). ROC curve analysis of the K_i change between baseline and 12 months in NAWM predicted a loss of NEDA status at 2 years with 86% sensitivity and 86% specificity (AUC 0.98, $p = 0.002$).
Conclusion: BBB permeability predicted alemtuzumab efficacy at two years, indicating that BBB permeability is a biomarker of treatment response in RRMS.

1. Introduction

Deciding the optimal treatment for relapsing-remitting multiple sclerosis (MS) is challenging, as whilst early effective disease control reduces the risk of permanent disability (Harding et al., 2019), many of the highly efficacious treatments have potentially serious adverse effects. Alemtuzumab, a monoclonal anti-CD52 antibody, is a high-efficacy disease-modifying treatment that specifically depletes T- and B-lymphocytes, significantly reducing the annualised relapse rate in MS patients, but with a high risk of long-term autoimmune complications such as thyroid disfunction (Coles et al., 2011; Kalincik et al., 2017).[1]

Traditional MRI measures of disease severity correlate poorly with long-term disability accumulation (Fahrbach et al., 2013; Kappos et al., 1999), and more recent imaging markers of disease progression such as global or regional atrophy are not yet recommended for monitoring at the patient level (Sastre-Garriga et al., 2020).

The blood–brain barrier (BBB) is compromised in multiple sclerosis,

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[1] NEDA: No evidence of disease activity. EDSS: Expanded disease severity score. DCE-MRI: Dynamic contrast-enhanced magnetic resonance imaging. BBB: Blood–brain barrier. NAWM: Normal appearing white matter. GM: Gray matter. BPF: Brain parenchymal fraction.

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its permeability can be quantified by dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) using a validated protocol (Varatharaj et al., 2019) and this has been shown to predict the treatment response to natalizumab and fingolimod, and the risk of conversion from optic neuritis to definite MS (Cramer et al., 2018, 2015; Cramer SP Frederiksen JL, Larsson HBW, 2016; Ortiz et al., 2014). We hypothesized that BBB integrity measured by MRI can be used as an early marker of treatment efficacy after alemtuzumab, specifically No Evidence of Disease Activity-3 (NEDA-3) status two years after treatment initiation.

The aim of the present study was to investigate the effect of alemtuzumab on the leakiness of the BBB and the predictive capabilities of individual BBB responses on subsequent disease activity.

2. Materials and methods

Further details available in Supplementary Materials and Methods.

2.1. Participants

Study participants were prospectively recruited through the multiple sclerosis clinic at Copenhagen University Hospital - Rigshospitalet, Denmark. The inclusion criteria were as follows: 1) relapsing-remitting multiple sclerosis according to the McDonald 2010 criteria (Polman et al., 2011), 2) age 18–60 years, 3) indication for alemtuzumab treatment and 4) physically and mentally able to participate in a study. The exclusion criteria were 1) contraindications for MRI scanning, 2) kidney disease, 3) other neurological disorders in the same patient, and 4) previous reactions to MR contrast agent, bronchial asthma or history of other severe allergies. The treatment was administered intravenously in two courses 12 months after each other, with five days of consecutive infusions of 12 mg in the first coure and three consecutive days of 12 mg in the second. Baseline clinical characteristics were collected from medical records. The clinical end point, no evidence of disease activity (NEDA-3) status, was determined from MRI T2 lesion activity, EDSS score and relapse data collected from medical records at two years from treatment initiation as described elsewhere (Rotstein et al., 2015).

2.2. Imaging

MRI was performed on a 3T magnetic resonance unit (Achieva dStream; Philips, Best, the Netherlands). Patients underwent MRI before and 6, 12, and 18 months after the first course of alemtuzumab. The 12-month scan was carried out prior to the second course of alemtuzumab. DCE-MRI and T2 FLAIR sequences were recorded as described elsewhere, except the total dose of gadobutrol was 0.090 mmol/kg body weight, administered as two boluses after the 15th and 80th time points (Cramer et al., 2014). ROIs were placed at a minimum of 10 mm from T2 lesions.

Anatomical images for tissue segmentation were acquired as a 3D T1 weighted turbo field echo sequence. Volumetric segmentation and calculation of brain parenchymal fraction was performed with the standard longitudinal stream of FreeSurfer image analysis suite (FreeSurfer 7.1.0 Fischl et al., 2002, Reuter et al., 2012).

2.3. Blood–brain barrier permeability assessment

The DCE-MRI data were analysed with a semiautomated procedure as previously described (Larsson et al., 2009), blinded to the NEDA-3 status of the patients. Fig. 1 summarizes the method.

The median signal–time curve for all voxels in the ROI was extracted (Fig. 1D) and used to calculate the permeability as previously described (Cramer et al., 2015; Cramer and Larsson, 2014). Tissue concentration–time curves were evaluated using a Patlak model (Fig. 1E), as described in a previous work (Larsson et al., 2009). Values of K_i are reported as ml/100 g/min, assuming a brain tissue density of 1 g/ml (Barber et al., 1970).

2.4. Statistics

Statistical analysis was performed using SPSS 24, R version 4.0.3 and RStudio 1.4 (IBM Corp., 2016; Ozenne, 2021; Pinheiro et al., 2021; R Core Team, 2020; RStudio Team, 2020). Generalized least squares fitted linear models for Ki in NAWM, GM and the thalamus and for lymphocyte counts were calculated with the gls-function from the R package LMMstar version 0.1.11. Patients were set as random effects, disease status and time as fixed effects, and K_i as the outcome measure. Missing follow-up data timepoints on K_i points were excluded by default in the gls-models. For all analyses, $p < 0.05$ was considered statistically significant. Multiple comparisons were corrected for via false discovery rate (FDR) estimation. Receiver operating characteristic (ROC) curves calculated with SPSS were used to estimate the ability of K_i change from baseline to predict NEDA-3 status at two years and to establish a cut-off value providing the best combined sensitivity and specificity. The time points used for the ROC curve analyses of each tissue were those at which there was a statistically significant difference in change in *Ki* between the patient groups.

3. Standard protocol approvals, registrations, and patient consent

3.1. 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-1–2014–132. All experiments were conducted in accordance with the Declaration of Helsinki of 1975, and all subjects gave written informed consent.

3.2. Data availability

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials. Anonymized data used for this study are available upon request.

3.3. Trial registration

This research project is registered at ClinicalTrials.gov with identifier NCT03193086.

4. Results

4.1. Participants

The screening and inclusion procedure is described in Fig. 2. Fifteen patients were included in the final analyses. Four patients, two of which experienced disease activity and two of which had no evidence of disease activity within two years, did not complete the 18-month MRI scan. Eight patients (53%) lost their NEDA status within two years post-treatment (four due to the appearance of new or enlarged T2 lesions, four due to one or more clinical relapses and none from a sustained increase in EDSS). Baseline demographics and clinical characteristics at baseline were similar between the two patient groups (summarized in Table 1). Any previous disease-modifying treatment before alemtuzumab is summarized in Table 1. The baseline K_i in NAWM was significantly higher for the NEDA group compared to the disease activity group (0.0198 ml/100 g/min, $p = 0.028$) (Fig. 4).

4.2. Blood–brain barrier permeability change as a predictor of disease activity

K_i changes during alemtuzumab treatment can be seen in Fig. 4. Patients who maintained NEDA status at two years after treatment initiation had a significantly larger decrease in BBB permeability (K_i) in

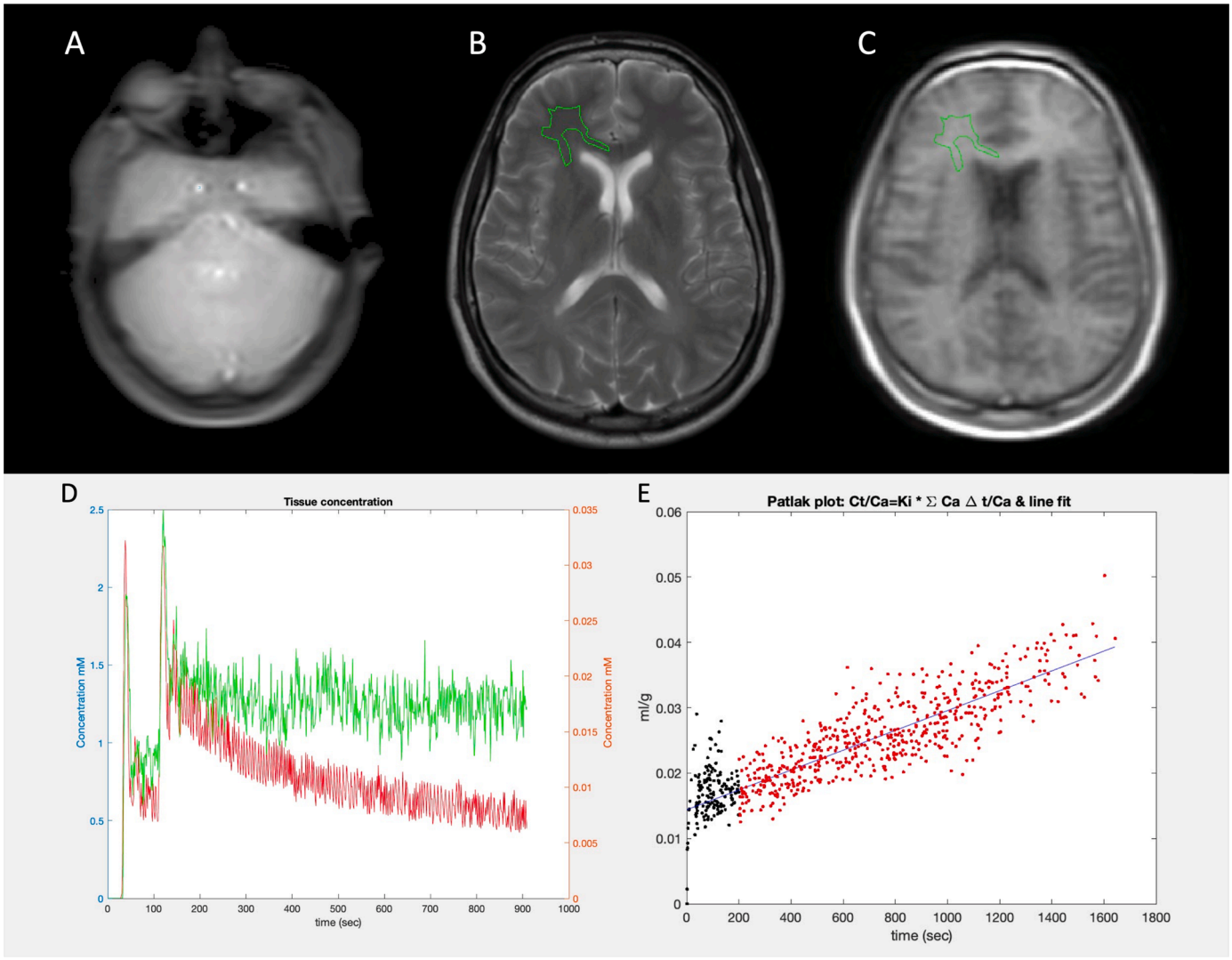

Fig. 1. Determination of K_i. A) Example of DCE slice where the arterial input function is derived from the highest intensity voxel of the internal carotid artery. B) Example of manually delineated ROI on T2 map. C) Example of transferred ROI from manually delineated T2-map to DCE slice. *D) Example of arterial input function (red) and tissue function (green) E) Example of Patlak-plot for Ki determination. Red indicates data points after the vascular phase, which are included in the linear regression.* K_i values in grey matter (GM) were interpolated from K_i and corresponding fraction of GM from tissue segmentation.

periventricular NAWM between baseline and the six-month follow-up than those who lost NEDA status (mean difference −0.029 ml/100 g/min, 95% confidence interval [CI] = −0.005 – −0.053 ml/100 g/min, p = 0.03) (Fig. 3 and 4, Supplementary results Table A). A significantly larger decrease in NAWM K_i was also observed between baseline and 12 months in the group that maintained NEDA (mean difference 0.043 ml/100 g/min, 95% confidence interval [CI] = −0.022 – −0.065 ml/100 g/min, p = 0.002) (Fig. 4). GM BBB permeability change between baseline and six months also predicted the NEDA status (mean difference −0.038 ml/100 g/min, 95% CI = −0.006 – −0.070 ml/100 g/min, p = 0.03); however, this was not the case in the thalamus (mean difference −0.017 ml/100 g/min, 95% CI = −0.003 – 0.047, p = 0.14) (Fig. 3). There was no significant difference in K_i between baseline and 18 months between the two groups for any of the tissues examined (Fig. 4).

Changes in lymphocyte counts did not predict NEDA status, and there was no significant correlation between changes in lymphocyte counts from baseline to six months for any of the tissues examined (Fig. 5). The development of *Ki* in NAWM over time colour coded by previous treatment can be seen in Supplementary results Figure D.

ROC curve analysis for treatment-associated BBB permeability changes in NAWM predicted disease activity within two years. The baseline-6 m NAWM K_i difference showed an AUC of 0.85 (p = 0.02), and a cut-off of −0.0178 yielded a sensitivity of 88% and a specificity of 76% (Fig. 6). The baseline-12 m NAWM K_i difference had a higher AUC of 0.98 (p = 0.002), with a cut-off of −0.0178 able to predict two-year NEDA status with a sensitivity of 88% and specificity of 86%. ROC curve analysis of GM K_i change baseline-6 m AUC showed a non-significant trend (AUC = 0.79, p = 0.06).

4.3. Brain parenchymal fraction (BPF)

In order to justify DCE-MRI (and therefore contrast administration) we investigated whether structural MRI changes (BPF and lesion volume change) could provide the same information. BPF decreased significantly from baseline to 6 months in the entire study group (−0.004, paired-samples *t*-test; p = 0.04). Neither baseline BPF nor the change in BPF from baseline to 6 months differed with NEDA status at two years (Supplementary Figure A). BPF did not correlate with K_i over all time points; however, the change in BPF from baseline to 6 months correlated with the change in K_i from baseline to 6 months (Spearman CC = 0.58, p = 0.023), and the change in BPF from 12 to 18 months correlated with the change in K_i during the same period (Spearman CC = 0.93, p =

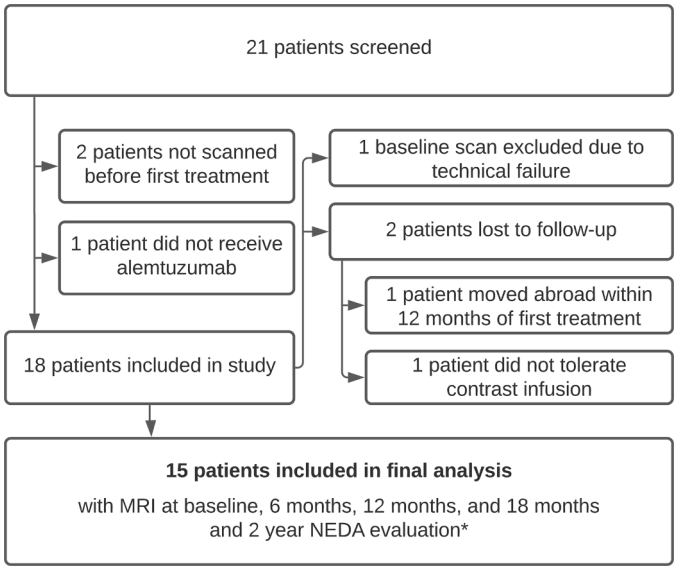

Fig. 2. Flowchart of patient recruitment. Study participants whose scans were included in the final analysis. Twenty-one patients were screened for the study, 18 patients were scanned before alemtuzumab treatment, and 15 patients were included in the final analysis. For two patients, it was not possible to carry out the MRI scan before the first alemtuzumab treatment, and one did not receive alemtuzumab due to low leucocyte counts. Of the 18 patients scanned before alemtuzumab treatment, one patient's baseline scan was excluded due to technical failure, and two patients were lost to follow-up (one due to non-tolerance of the administered MRI contrast agent, and one moved abroad within 12 months of receiving the first alemtuzumab infusion). Four patients (two from the disease activity group and two from the NEDA group) did not complete MRI at 18 months. All 15 patients completed the MRI scans at baseline, six months and 12 months which were used for NEDA prediction. *Of these, four subjects missed MRI at 18 months.

Table 1
Demographical and clinical data according to NEDA status 2 years after treatment initiation. P values for sex, methylprednisolone use and gadolinium-enhancing lesions were calculated with Fisher's exact test. The remaining p values were calculated with Student's *t*-test.

	NEDA lost (N = 8)	NEDA maintained (N = 7)	p value (raw)	p value (adjusted)
Age, years (mean; SD)	32.40 (8.2)	33.60 (7.2)	0.77	1
Female,%	75.0	85.7	1	1
EDSS score at baseline (median; range)	2.0 (0–3.0)	2.5 (0–6.5)	0.25	1
Methylprednisolone use < 60 days pretreatment, N	1	2	0.57	1
T2 lesion volume, ml (median; range)	7.42 (4.32–40.67)	12.55 (1.16–31.88)	0.94	1
≥1 Gd+ lesion at baseline, N	3	3	1	1
≥1 Gd+ lesion at six months, N	1	0	1	1
Previous most recent disease modifying treatment	4 fingolimod 4 natalizumab	2 natalizumab 3 fingolimod 1 interferon beta 1 dimethyl fumarate	NA	1
Days since treatment end (median; range)	42.5 (36–57)	46.0 (5–882)	0.37	1

0.00004) (Fig. 7, Supplementary Figure C). Neither BPF nor lesion volume changes from baseline to six months predicted NEDA status.

5. Discussion

Our results indicate that treatment-associated changes in BBB permeability can be used as a surveillance tool of treatment response. In the NEDA group, we observed a significant decrease in K_i from baseline to six months in both normal-appearing white matter (NAWM) and GM. Moreover, we also observed a significant decrease in K_i from baseline to 12 months in NAWM in the NEDA group. T2 lesion volume change and brain parenchymal fraction did not differentiate between NEDA and non-NEDA groups. This suggests that there may be a place for DCE-MRI in monitoring alemtuzumab treatment. One clinical scenario where this may help is in those patients who after the two courses, during subsequent follow-up imaging, demonstrate one new small lesion in the absence of clinical activity – in these patients there may be equipoise whether to give a third dose, and the 0–6 month K_i response may help inform this decision – this needs further study.

Considering the increasing awareness that GM is involved in MS pathology, it is interesting to note that the K_i decrease was also significant for GM in the NEDA group, suggesting that alemtuzumab treatment affects inflammation in the GM. The fact that we found a K_i decrease from baseline to both six and twelve months strengthens the validity of this change being related to a treatment effect of the first course of alemtuzumab.

We found a higher K_i at baseline in the NEDA group compared to the non-NEDA group. One may speculate that baseline K_i separates two groups of patients, with disease mainly driven by luminal (systemic) and abluminal (intrathecal) immune responses relating to the blood or brain side of the BBB respectively. Alemtuzumab is likely to suppress the luminal drivers (systemic inflammation, circulating lymphocytes) after the first infusion. Hence those with a more leaky BBB may be more likely to respond to alemtuzumab since in these patients the BBB leakiness may itself indicate that disease activity is driven by luminal factors (systemic inflammation, circulating lymphocytes). On the other hand, those with a less leaky BBB may have a more compartmentalized intrathecal immune response behind the BBB (abluminal) which is either inaccessible or unresponsive to alemtuzumab.

After the second course of alemtuzumab, administered at 12 months, we observed an increase in NAWM K_i back to baseline in the group that retained NEDA status. This did not mirror the lymphocyte counts, which decreased after each infusion course (Fig. 5). This suggests that BBB permeability to a soluble agent such as gadolinium may not necessarily be linked to peripheral lymphocytes available for trafficking across the BBB, and is more likely to be linked to a combination of other factors such as microvascular structural changes and inflammatory signalling. A dissociation between cellular trafficking and solute permeability has previously been observed in vitro, where interferon beta was found to reduce lymphocyte transmigration, while no effect was observed on the permeability to albumin (Prat et al., 2005).

The decrease in BPF from baseline to 6 months following alemtuzumab initiation observed in this study is in line with what has been observed in other MS cohorts 6–12 months after initiation of anti-inflammatory treatment (Portaccio et al., 2013; Vidal-Jordana et al., 2013). These apparent changes in brain volume are hypothesized to reflect treatment-related resolution of neuroinflammation and oedema, thus resulting in a reduction in the tissue water content and perhaps, more importantly, changes in the inflammatory cell volume, particularly microglial cells (De Stefano and Arnold, 2015). This phenomenon has previously been named "pseudoatrophy" to differentiate it from true atrophy, i.e. irreversible brain tissue loss. Several DMT studies have found that the accelerated decrease in brain volume following treatment initiation is higher in the presence of gadolinium-enhancing lesions at baseline (Sastre-Garriga et al., 2015; Vidal-Jordana et al., 2013). One study found that following natalizumab treatment, patients with

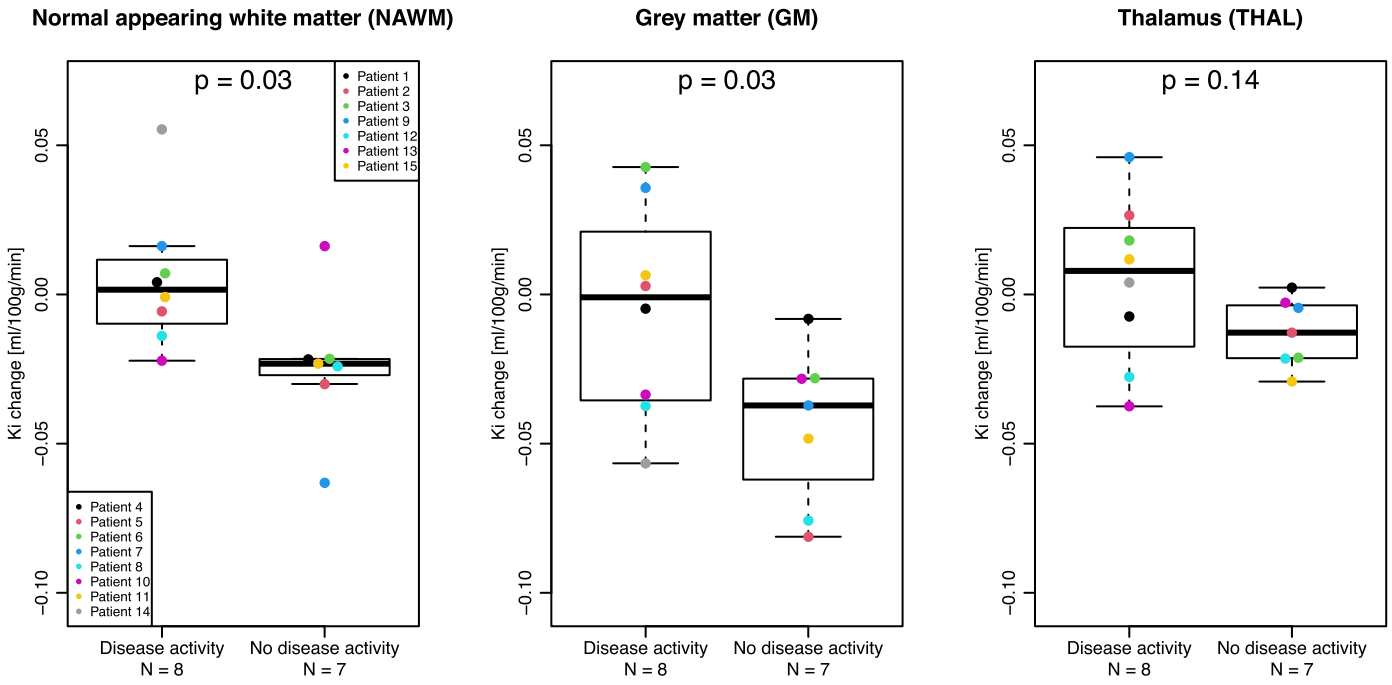

Fig. 3. Treatment associated blood-brain barrier (BBB) permeability change from baseline to six months in normal appearing white matter (NAWM) and grey matter (GM) predicts disease activity (NEDA status) within two years. Ki values in grey matter were derived from automatic segmentation of grey matter and linear regression model approach. Ki in NAWM and thalamus were obtained from manual placing of ROIs. The change in the thalamus does not predict disease activity. Boxes represent median and interquartile range with whiskers at 1.5 times the inter-quartile range, dots represent individual measurements.

gadolinium-enhancing lesions at baseline showed a greater degree of decrease in brain volume up to 24 months after therapy initiation, indicating that therapy related brain volume changes can continue beyond one year (Sastre-Garriga et al., 2015). Thus, the correlation between K_i and BPF observed after both infusions of alemtuzumab and occurring irrespective of NEDA status supports the notion of K_i as a marker of subclinical neuroinflammation in MS.

In this study, 47% of patients maintained their NEDA status at two years. This is slightly higher compared to previous studies, specifically 39% in one study where alemtuzumab was the first line treatment (Cohen et al., 2012; Coles et al., 2011) and 32% with alemtuzumab as the second line treatment (Center et al., 2012). Another study reported that EDSS remained stable in 68% of patients, and 75% of patients were relapse-free at two years, but did not report the combined NEDA status (Theodorsdottir et al., 2021). Finally, one study found that 80% stayed free from relapses and 93% stayed free from disability accumulation at two years (Kalincik et al., 2017). However, these studies did not include MRI lesions, which could account for the contrast with our study, as there is substantial subclinical disease activity in RRMS, and half of the patients in our current study lost NEDA status due to new MRI activity. Previous evaluation of NEDA as a predictor of long-term outcome has shown that for patients with MS, having NEDA at two years gave a positive predictive value of 78.3% for the absence of progression at seven years (Rotstein et al., 2015). Thus, NEDA status at two years may be a good prognostic marker of long-term disease progression.

Interpretation of this study's findings are limited by the small sample size of 15 patients. Between 2009 and 2019, only 167 RRMS patients in Denmark received alemtuzumab for ≥2 year. (Theodorsdottir et al., 2021). Previous studies have included more patients (29 patients (Gilmore et al., 2020), 85 (Harding et al., 2019), 189 (Kalincik et al., 2017), 215 (Coles et al., 2011)), but implementation of article 20 from the European Medical Agency restricted the use of alemtuzumab (European Medicines Agency, 2019), and consequently the pool of potential patients for the study resulted in reduced recruitment. Despite the relatively low number of study participants, the NEDA group and the disease activity group were similar in the clinical aspects examined, such as age, sex, prestudy treatment and methylprednisolone use.

In this study, we placed ROIs in WM away from T2 lesions to achieve a measure of lesion-free normal-appearing white matter. However, lesion detection and extraction were not possible in GM since we lacked the appropriate MRI sequence. As the decrease in K_i was also significant in GM for the NEDA group, future studies of K_i in GM are encouraged to include GM lesion segmentation, for instance, by GM lesion visualization using a double inversion recovery sequence. Furthermore, K_i measurements in this study may be biased by a difference in prestudy treatments between the patients since treatments may have differing effects, including rebound effects on BBB permeability. However, there was a similar pre-treatment distribution of the two most common disease-modifying treatments in this study, natalizumab and fingolimod, as well as methylprednisolone use between the groups.

We have previously found that K_i in NAWM at six months after treatment initiation of either fingolimod or natalizumab is a predictor of NEDA status at two years, while the change in K_i from baseline to 6 months was not related to NEDA status at two years. This may have been because of baseline methylprednisolone use in this study, which was found to be a significant predictor of baseline K_i (Cramer et al., 2018). Moreover, compared to fingolimod and natalizumab, alemtuzumab causes a profound non-selective depletion of lymphocytes (Baker et al., 2017), and hence a change in K_i might have been more likely to be detected in the present study.

6. Conclusion

Here we report evidence that BBB permeability during alemtuzumab treatment can be used as a predictor of treatment efficacy, as measured by NEDA-3 status at two years. This validates previous research indicating that BBB permeability is a marker of treatment response in relapsing-remitting multiple sclerosis.

Declaration of Competing Interest

The author(s) declared the following potential conflicts of interest

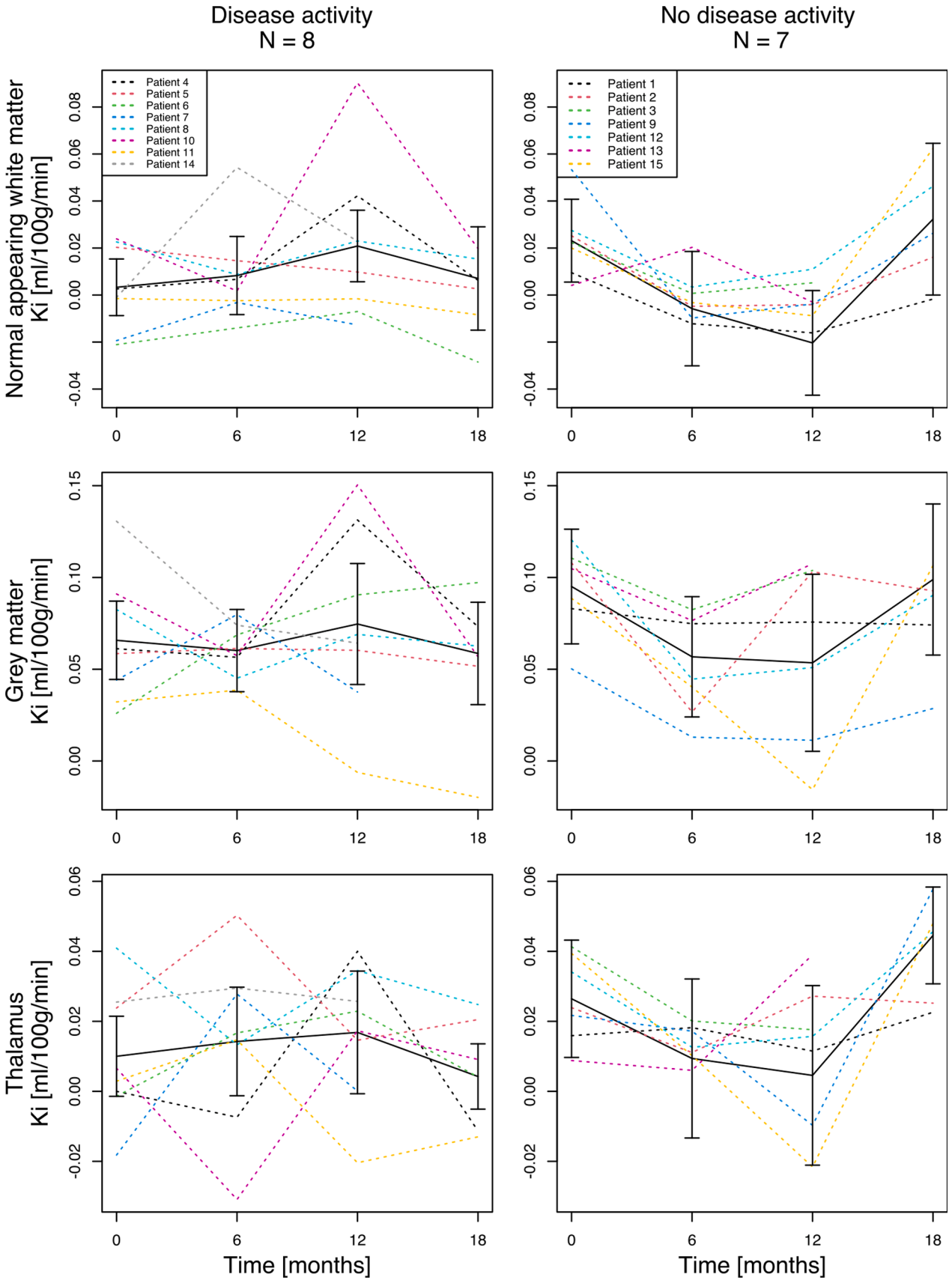

Fig. 4. Blood–brain barrier (BBB) permeability development over the course of 18 months of alemtuzumab treatment, showing an early decrease in treatment responders (NEDA maintained) as opposed to treatment non-responders (NEDA lost). Solid lines represent group averages with standard errors, dashed lines represent measurements of individual patients.

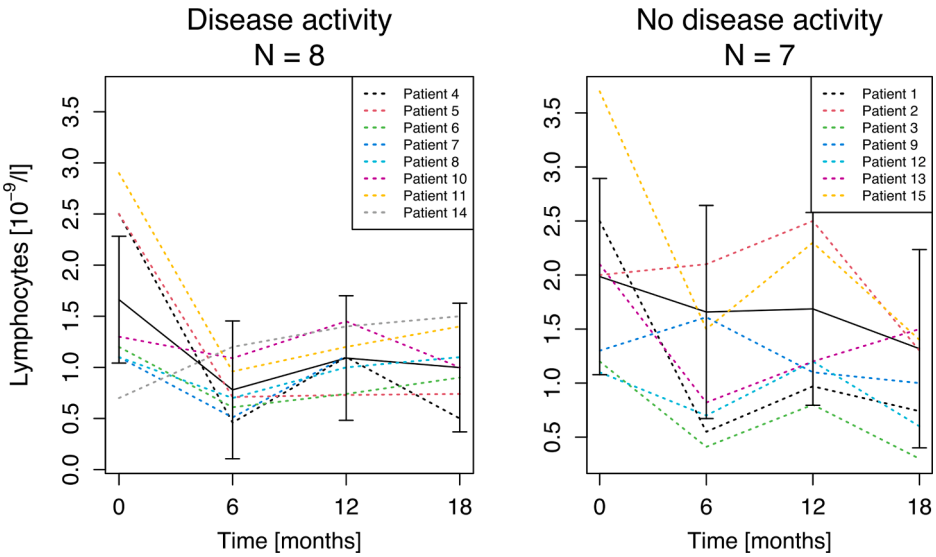

Fig. 5. Blood lymphocyte counts over time according to NEDA status (disease activity yes/no) at two years from treatment initiation. Solid lines represent group averages with standard errors, dashed lines represent measurements of individual patients.

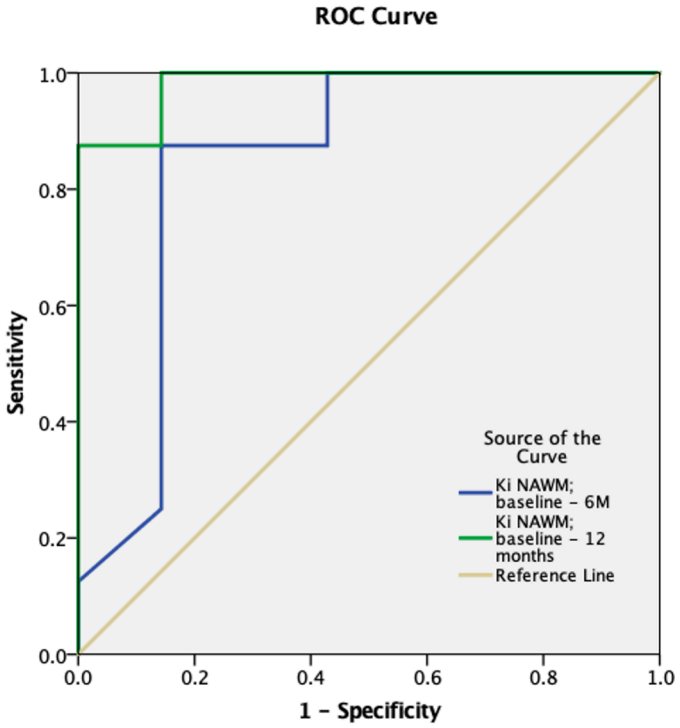

Fig. 6. ROC curve for treatment-associated blood–brain barrier (BBB) permeability changes in normal appearing white matter (NAWM) predicts disease activity within two years. Baseline-6 m NAWM K_i difference AUC = 0.85, p = 0.03. Cut-off −0.0178: sensitivity 88%, specificity 76%. Baseline-12 m NAWM K_i difference AUC = 0.98, p = 0.006. Cut-off −0.0178: sensitivity 88%, specificity 86%. ROC curve analysis of grey matter K_i change baseline-6 m AUC was not significant (AUC = 0.79, p = 0.06).

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M.H. Knudsen: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Investigation, Writing - Original Draft, Writing - Review & Editing, Visualization, Project administration

U. Lindberg: Methodology, Software, Formal analysis, Writing - Review & Editing, Supervision

J.L. Frederiksen: Conceptualization, Investigation, Resources, Writing - Review & Editing, Supervision, Funding acquisition

M.B. Vestergaard: Methodology, Writing - Review & Editing, Supervision

H.J. Simonsen: Methodology, Investigation, Writing - Review & Editing

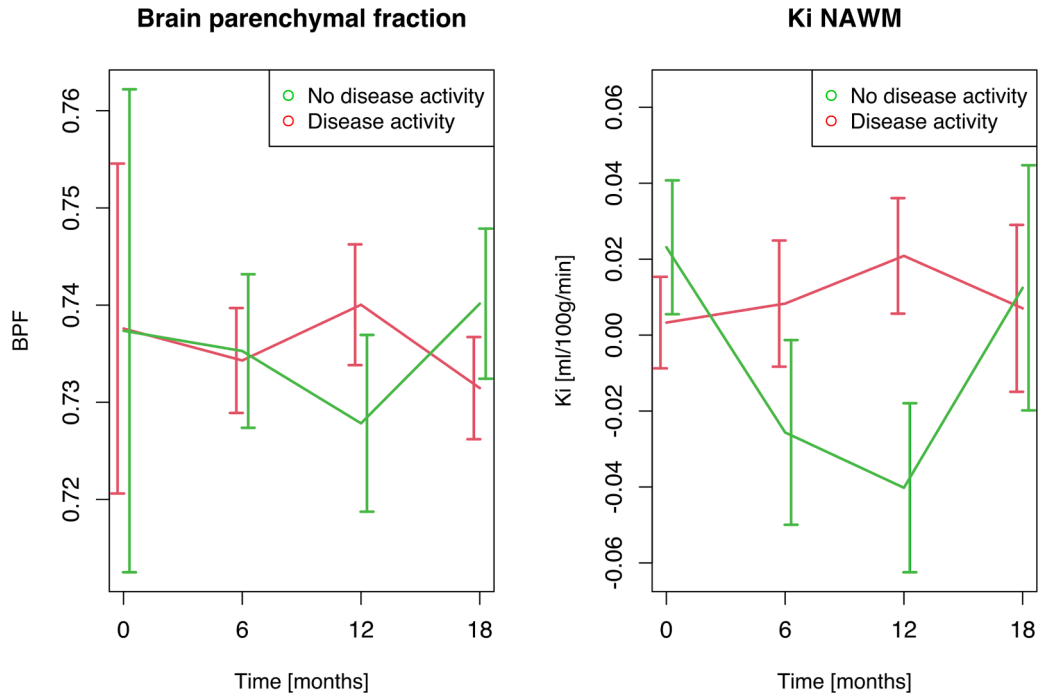

Fig. 7. Brain parenchymal fraction and K_i in normal appearing white matter (NAWM) develops similarly across time. Green lines: mean and standard errors of the No disease activity group (NEDA maintained). Red lines: mean and standard errors of the Disease activity group (NEDA lost).

A. Varatharaj: Conceptualization, Writing - Review & Editing

I. Galea: Conceptualization, Writing - Review & Editing,

M. Blinkenberg: Investigation, Resources, Writing - Review & Editing

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H.B.W. Larsson: Conceptualization, Methodology, Software, Formal analysis, Resources, Writing - Review & Editing, Visualization, Supervision, Funding acquisition

S.P. Cramer: Conceptualization, Methodology, Software, Validation, Formal analysis, Resources, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision, Project administration, Funding acquisition

Conceptualization	Ideas; formulation or evolution of overarching research goals and aims
Methodology	Development or design of methodology; creation of models
Software	Programming, software development; designing computer programs; implementation of the computer code and supporting algorithms; testing of existing code components
Validation	Verification, whether as a part of the activity or separate, of the overall replication/ reproducibility of results/ experiments and other research outputs
Formal analysis	Application of statistical, mathematical, computational, or other formal techniques to analyse or synthesize study data
Investigation	Conducting a research and investigation process, specifically performing the experiments, or data/evidence collection
Resources	Provision of study materials, reagents, materials, patients, laboratory samples, animals, instrumentation, computing resources, or other analysis tools
Data Curation	Management activities to annotate (produce metadata), scrub data and maintain research data (including software code, where it is necessary for interpreting the data itself) for initial use and later reuse
Writing - Original Draft	Preparation, creation and/or presentation of the published work, specifically writing the initial draft (including substantive translation)
Writing - Review & Editing	Preparation, creation and/or presentation of the published work by those from the original research group, specifically critical review, commentary or revision – including pre-or postpublication stages
Visualization	Preparation, creation and/or presentation of the published work, specifically visualization/ data presentation
Supervision	Oversight and leadership responsibility for the research activity planning and execution, including mentorship external to the core team
Project administration	Management and coordination responsibility for the research activity planning and execution
Funding acquisition	Acquisition of the financial support for the project leading to this publication

Declaration of Competing Interest

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Supplementary materials

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