Supplemental material

Methods

MRI acquisition

High field MRIs were acquired using a 3T Siemens whole body scanner (Trio TIM; Siemens, Erlangen, Germany) by applying a 12-channel receive head coil. The imaging protocol included pre- and post contrast 3-dimensional T1*w MPRAGE (echo time = 3.03 milliseconds [ms], repetition time = 1900 ms, spatial resolution = (1×1×1) mm^3, Gadolinium (0.1mmol Gd/kg) was used as contrast agent), and a 3-dimensional fluid attenuated inversion recovery (FLAIR, echo time = 388.0 ms, repetition time = 6000.0 ms, inversion time = 2100 ms, spatial resolution = (1×1×1) mm^3). In addition, a 3-dimensional gradient echo flow-compensated susceptibility weighted imaging (SWI) (echo time = 28 ms, repetition time = 35 ms, flip angle = 15°, spatial resolution = (1×1×1) mm^3) yielding magnitude, SWI-filtered phase and reconstructed SWI images was acquired.

SWI image analysis

MRI scans were analyzed by investigators that were trained to assess SWI lesion morphology (NB, EB) and an investigator with experience in the analysis of SWI images (TS) blinded to clinical details (diagnosis, EDSS, age, sex) using the 3D Slicer software package (version 4.6; The Slicer Community, http://www.slicer.org) after co-registration of FLAIR images to SWI. In a first step, the investigators determined the lesion count on 3D FLAIR images (FLAIR hyperintense lesions with a diameter of >3mm were selected). Each FLAIR lesion was then looked up on co-registered SWI to describe the existence of a SWI hypointense core or a SWI hypointense ring at the edge of the lesion at baseline. Contrast enhancing lesions with a SWI hypointense ring were classified as “acute ring lesions”, and non-contrast enhancing lesions with a SWI

hypointense ring were classified as “chronic ring lesions”. Next, changes in lesion morphology were noted at follow up.

Additional image analyses

Contrast enhancement, and the number of new lesions at follow up were assessed manually by trained investigators (NB, EB) blinded to clinical details (diagnosis, EDSS, age, sex) using the 3D Slicer software package (version 4.6; The Slicer Community, http://www.slicer.org). Lesions were automatically segmented by using a pre-trained deep learning algorithm based on multi-dimensional gated recurrent units (GRU). Next, lesion masks were manually corrected by trained investigators (NB, EB, and TS) at follow up and baseline to finally calculate lesion volumes. Brain volume changes were estimated by calculating percentage of brain volume change (PBVC) using Structural Image Evaluation using Normalization of Atrophy (SIENA) with optimized parameters for brain extraction.[20] The normalized mean intralesional T1w signal intensity was calculated by division of /mean intralesional T1w signal intensity/ by /mean normal appearing white matter T1w signal intensity/. Shrinking lesions were defined as lesions with a decrease in FLAIR volume of at least 50% and 25 voxel.

Statistical analysis

MSFC scores were calculated via the formula MSFC ={ (average (1/9HPT) - baseline mean (1/9HPT) / baseline standard deviation (1/9HPT) + { - (average 25FWT - baseline mean 25FWT) / baseline standard deviation 25FWT} + (PASAT - baseline mean PASAT) / baseline standard deviation PASAT} / 3.0.

Results

SWI lesion morphology of Gadolinium enhancing lesions at baseline

Gadolinium enhancement was studied in 579 lesions at baseline. 33 of these 579 lesions (6%) showed Gadolinium enhancement at baseline. In comparison to non-Gadolinium enhancing lesions, Gadolinium enhancing lesions more often had a SWI hypointense core (10 lesions (30%) versus 40 of 546 (7%) non-Gadolinium enhancing lesions, $p<0.001$). Furthermore, 5 lesions (15%) showed a SWI hypointense ring ($p=0.02$). Those lesions were classified as “acute ring lesions”. Finally, two lesions (6%) had diffuse SWI hypointense areas.

Association of SWI lesion morphology at baseline with lesion size and T1w signal intensity at baseline

Hypointense ring lesions without contrast enhancement (“chronic hypointense ring lesions”) were larger (median FLAIR lesion volume 304mm^3, range 132-1271mm^3) and had lower normalized T1w intensity values (median normalized T1w intensity 0.74, range 0.50-1.01) in comparison to lesions without a ring (median FLAIR lesion volume 82mm^3, range 4-2000mm^3, MWU, $p<0.001$; median normalized T1w intensity 0.90, range 0.01-1.21, MWU, $p<0.001$).

In contrast, we did not observe differences in lesion size (MWU, $p=0.472$) nor normalized T1 intensity values (MWU, $p=0.748$) between lesions with and without a SWI hypointense core.

Association of SWI imaging markers at baseline with MRI measures at baseline

In total, 20 patients (30%) had SWI hypointense core lesions. We did not observe statistical significant differences in age, gender, disease duration or treatment between patients with and without SWI hypointense core lesions (table 1). Patients with SWI hypointense core lesions had more FLAIR lesions (MWU, $p<0.001$, table 1), higher lesion volumes (MWU, $p<0.001$, table 1), and more contrast enhancing lesions (MWU, $p=0.022$) at baseline. Accordingly, the number of SWI hypointense core lesions correlated with the number of FLAIR lesions (Spearman, $p<0.001$, $rho=0.507$), FLAIR lesion volume (Spearman, $p<0.001$, $rho=0.522$), and the number of contrast enhancing lesions (Spearman, $p=0.014$, $rho=0.301$) at baseline. The proportion of SWI hypointense core lesions correlated with the number and volume of FLAIR lesions (Spearman, lesion count $p=0.004$, $rho=0.361$, lesion volume $p=0.001$, $rho=0.430$). We did not observe an association between the number of SWI hypointense core lesions and the mean normalized intralesional T1w signal intensity (Spearman, $p=0.697$, $rho=-0.053$).

Moreover, 13 patients (19%) had SWI hypointense ring lesions. Of these 13 patients, 7 patients also had hypointense core lesions. We did not observe significant differences in age, gender, disease duration or treatment between patients with and without SWI hypointense ring lesions (table 1). Patients with SWI hypointense ring lesions had more FLAIR lesions (MWU, $p<0.001$), higher lesion volumes (MWU, $p<0.001$), and more contrast enhancing lesions (MWU, $p=0.002$) at baseline. Accordingly, the number of SWI hypointense ring lesions correlated with the number of FLAIR lesions (Spearman, $p<0.001$, $rho=0.467$), FLAIR lesion volume (Spearman, $p<0.001$, $rho=0.514$), and the number of contrast enhancing lesions (Spearman, $p=0.001$, $rho=0.407$) at baseline. The proportion of SWI hypointense ring lesions

correlated with the number and volume of FLAIR lesions (Spearman, lesion count p=0.001, rho=0.402, lesion volume p<0.001, rho=0.479). Also, the number of SWI hypointense ring lesions inversely correlated with the mean normalized intralesional T1w signal intensity (Spearman, p=0.013, rh=-0.328), and patients with SWI hypointense ring lesions had lower normalized intralesional T1w signal intensity values (MWU, p=0.014).

Association of SWI imaging markers at baseline with clinical parameters at baseline

The number of SWI hypointense ring lesions per patient correlated with lower SDMT values (Spearman, p=0.032, rho=-0.316). We did not observe an association between the number of SWI hypointense core or ring lesions and any other clinical measures including sex, age, disease duration, EDSS, SDMT, MSFC, 9HPT, T25FWT, and PASAT (all p > 0.112). Accordingly, Patients with SWI hypointense core or ring lesions, did not differ regarding clinical measures (all p > 0.091, table 1) except for patients with SWI hypointense ring lesions who had lower SDMT values (MWU, p=0.031).

Prediction of MRI parameters at follow up by SWI imaging markers at baseline

The number of SWI hypointense core lesions per patient at baseline correlated with the degree of brain atrophy during follow up (PBVC, Spearman, p<0.001, rho=-0.424), but did not predict the number of new lesions (Spearman p=0.932, rho=0.011) or the degree of mean normalized T1w signal intensity (Spearman, p=0.924, rho=-0.012) at follow up. Accordingly, patients with SWI hypointense core lesions at baseline had higher brain atrophy rates during follow up (MWU, p=0.008, table 1), but not more new (MWU, p=0.750, table 1) or more T1w hypointense lesions (MWU, p=0.062).

The number of SWI hypointense ring lesions at baseline did not predict the degree of brain atrophy during follow up (PBVC, Spearman, p=0.333, rho=-0.121), the number of new lesions (Spearman p=0.895, rho=0.017) or the degree of mean normalized T1w signal intensity (Spearman, p=0.126, rho=-0.193) at follow up. Accordingly, patients with SWI hypointense ring lesions at baseline did not have higher brain atrophy rates, or more new or more T1w hypointense lesions (MWU, all p>0.150, table 1).

By using a general linear model with backward selection and PBVC as the dependent variable, and sex, age, disease duration, disease course, EDSS at baseline, lesion count, number of contrast enhancing lesions, and SWI hypointense rim/core lesions as independent variables, only sex (B=0.261, p=0.027) remained significant within the model. Lesion count and SWI hypointense core lesions were intercorrelated.

By using two general linear models with backward selection and number of new lesions or T1w signal intensity at follow up as the dependent variable, and the same variables as in the previous model as independent variables, no variable was identified to explain variations in the dependent variables.

Predictive value of SWI imaging markers at baseline: clinical parameters

To further confirm the association between number of SWI hypointense ring lesions at baseline and EDSS at follow up, we build a general linear model with EDSS at follow up as the dependent variable, and sex, age, disease duration, disease course, EDSS at baseline, lesion count, lesion volume, number of contrast enhancing lesions, and SWI hypointense ring/core lesions as independent variables. The model predicted EDSS at follow up (p<0.001). Only the number of SWI hypointense ring lesions (p=0.018) and EDSS at baseline (p<0.001) significantly contributed to the model.

Importantly, the overall FLAIR lesion number (p=0.898) or volume (p=0.562) did not significantly contributed to the model.

Tables

Supplemental table 1. Missing values.

Variable	Missing values
EDSS at baseline	2 patients (3.0%)
EDSS at follow up	1 patients (1.5%)
MSFC at baseline and/or follow up	22 patients (33.3%)
SDMT at baseline and/or follow up	24 patients (36.3%)
Lesion mask at baseline and/or follow up (failed segmentation)	91 lesions (14.9%)

Supplemental table 1. Missing values.