Characteristics, prevalence, and clinical relevance of juxtacortical paramagnetic rims in patients with multiple sclerosis

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Supplemental data

eMethods

Clinical study

To assess the prevalence and clinical significance of JPRs, we screened quantitative susceptibility mapping (QSM) maps (obtained from segmented 3D-Echo Planar Imaging -3D-EPI-, 670μm isotropic, TR=64ms, TE=35ms), as well as MP2RAGE (1 mm^3 isotropic, TR=5000ms, TI1=700, TI2=2500ms) and 3D-FLAIR (1 mm^3 isotropic, TR=5000ms, TE=386ms, TI=1800ms) images of 165 MS patients of an ongoing monocentric cohort study (INsIDER) at the University Hospital of Basel (Basel, Switzerland) in order to assess for the presence of JPRs (QSM), PRLs (QSM), WMLs (FLAIR) and CL count/CL volume -CLV- (MP2RAGE).

The inclusion criteria for the INsIDER study were: (i) Multiple Sclerosis diagnosis according to McDonald criteria 2017[1] and diagnosis of active RRMS or inactive PMS as defined by Lublin et al.[2]; (ii) absence of severe psychiatric condition or neurological disease (excluding headache); other than MS (iii) absence of contraindication to MRI (e.g. claustrophobia, pacemaker). Progressive MS patients were non-active (i.e., no relapses and no MRI activity) and relapsing-remitting MS patients were active but the MRI was performed with a 3-month delay from the last relapse. All scans were performed on a 3T MRI whole-body MRI scanner (Magnetom Prisma, Siemens Healthineers, Erlangen, Germany).

JPRs were identified independently by two experienced neurologists (RG and AC), followed by consensus review. Serum samples for measurement of serum neurofilament light chain (sNfL) were collected at the time of the MRI (±3 months) in 123/165 patients. sNfL Z-scores were calculated as described by Benkert et al.[3]. Since BMI values where missing in our data, a BMI of 25 was used.
108 (98 without JPRs and 10 with JPRs) of the patients had a longitudinal standardized EDSS assessment in the context of the Swiss Multiple sclerosis cohort study (SMSC) (follow-up time range: 3.6 - 9 years, with average follow-up 6.1 years). These MS patients were comparable with the whole original cohort. (Supplementary data, table S1).

We tested the following H0-hypothesis: MS patients with JPRs do not differ from patients without JPRs in demographics (age and sex), disease duration, clinical phenotype, EDSS at time of the MRI and EDSS evolution over time, sNfL Z-scores, n. PRL, n. WML, n. CL and total CLV.

To test this hypothesis, we used a chi-square test for categorical data and a t-test for normally distributed continuous data. For non-normal data a Wilcoxon rank-sum test was used. (Table 1). To evaluate the disease progression rate in the two groups, we performed a Kaplan Meier estimator for time to first worsening by using the above-mentioned data from the SMSC. In addition to univariate analyses, we studied the association between presence of JPRs and total CLV (independent variable) in a logistic regression model, which was adjusted for age, disease duration and EDSS.

In a subgroup of patients (97 patients, 89 without JPRs and 8 with JPRs), a follow-up MP2RAGE after 2 years (±3 months) was available. For those patients, we calculated the rate of cortical thinning over this follow-up period with FreeSurfer[4] (version 6.0). We then examined changes in cortical thickness (CT) as follows: (CT timepoint 1 – CT timepoint 2) / CT timepoint 1 *100. The percentage change in cortical thickness was compared between patients with and without JPRs using a Wilcoxon rank-sum test.

The study follows the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guideline for reporting observational studies[5].

Postmortem imaging and histology

Five whole brains from deceased MS patients (3 with progressive MS -PMS- and 2 with relapsing-remitting MS -RRMS-, age: 62±7 years, disease duration: 20±12 years) were obtained from the MS Brain Bank and imaged on a clinical 3T whole-body MR system using a brain container filled with perfluoropolyether (Supplementary data, Table S2). The brains were fixed in 10% formalin within 24 hours from death and MRI was performed 3 to 12 months after fixation. MRI was acquired with the following sequences, adapted to ex vivo conditions: (i) segmented 3D-EPI to enable quantitative susceptibility mapping (QSM)[6] and (ii) MP2RAGE[7].

After imaging, brains were cut by using the state-of-the-art approach proposed by Luciano et al.[8]. Under MRI-guidance, we selected and excised tissue blocks containing JPRs from the brain slabs, which were then embedded in paraffin. Slices of 4 µm thickness were stained immunohistochemically using an avidin–biotin technique. Primary antibodies comprised anti–myelin basic protein (for myelin) and anti-CR3/43 (for MHC II positive microglia/macrophages). Moreover, we performed DAB-enhanced Turnbull staining for iron.

eReferences

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eTables

eTable 1: Clinical and demographical information of patients included in the Swiss Multiple Sclerosis Cohort (SMSC)

	Without JPR		With JPR		p-value
Number of. patients	98		10		
Age (years, median [IQR])	48.50	[36.00, 58.00]	45.0	[33.25, 53.75]	0.340
Sex = male (%)	41	(41.8)	3	(30.0)	0.698
Diagnosis = PMS (%)	43	(43.9)	2	(20.0)	0.262
EDSS.score (median [IQR])	3.0	[2.0, 4.5]	2.5	[1.5, 5.5]	0.733
MSSS (median [IQR])	4.30	[2.47, 5.87]	4.75	[3.02, 5.74]	0.890
Disease duration (median [IQR])	6.39	[1.30, 17.92]	5.24	[4.05, 17.78]	0.907

eTable 2: Postmortem study, patients' characteristics

Patient	Age	Sex	Disease course	Disease duration	EDSS	Comorbidities
1	65	F	SPMS	14 years	7,5	Restless legs syndrome, status after urothelial carcinoma, aortic insufficiency (grade 1-2), vitamin B12 deficiency, depression, cervical stenosis
2	51	F	RRMS	8 years	4	Non-small cell lung cancer, arterial hypertension, depression
3	58	M	SPMS	23 years	8	Restless legs syndrome
4	66	M	RRMS	17 years	2,5	Stenting for vertebral artery stenosis right, vertebral artery occlusion left, epilepsy
5	69	F	SPMS	39 years	8	Percutaneous nephrectomy owing to shrink kidney, symptomatic epilepsy, frontal meningioma, diabetes mellitus type II, arterial hypertension, coronary artery disease, status after PTCA and by-pass, vitamin D deficiency

F = female; M = male; RRMS = relapsing– remitting MS; SPMS = secondary progressive

MS; EDSS = Expanded Disability Status Scale;

eTable 3: Cortical thickness change over 2 years (±3 months) in all patients with available data (without JPRs vs. with JPRs)

	Without JPRs	With JPRs	p-value
No. patients	89	8	
Median rate of cortical thickness change	- 0.19%	-0.20%	0.811
Cortical thickness time point 1 (mm, mean)	2.337	2.359	0.685
Cortical thickness time point 2 (median number of voxels)	2.331	2.334	0.733