Supplementary information

Integration of magnetic resonance imaging and protein and metabolite CSF measurements to enable early diagnosis of secondary progressive multiple sclerosis

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MRI studies

MRI was performed at 1.5 T using the same imager and imaging protocol in all examinations. Gadopentetate dimeglumine (Magnevist®, 0.4 ml/kg body weight, *i.e.* double dose) was used as a contrast agent. MR images were analyzed visually. In the brain, T2 lesions >3 mm in diameter were counted in fluid attenuated inversion recovery (FLAIR) images using T2-weighted and proton density-weighted spin echo images as an aid. To account for differences in lesion volume, a 'T2 score' was created by weighting T2 lesions >10mm by a factor of three. T1 and Gd+ lesions were also counted. Large T1 lesions with a diameter >10mm and isodense to CSF were counted separately. Furthermore, T2 and Gd+ lesions were also counted in the spinal cord.

The width of the third ventricle was measured in coronal images. The sizes of the lateral ventricles and peripheral CSF spaces were graded from one to five according to a method described previously. Anteroposterior diameters of the spinal cord were measured in the sagittal plane at *vertebræ* C2, C5, Th3 and Th6.

Technical details of the MR examinations

Brain

Sagittal and transverse images were obtained with T2-weighted fluid attenuated inversion recovery sequences (FLAIR). Transverse and coronal images were acquired with T2-weighted turbo spin echo (SE) sequences. A transverse series was obtained with a proton density-weighted SE sequence. T1-weighted SE images were acquired in a transverse plane before contrast medium administration (gadopentetatedimeglumine, Magnevist®, 0.4 ml/kg body weight, i.e. double dose), and in transverse and coronal planes after that. Time between contrast medium injection and the first post-contrast T1-weighted series was about 10 min. The slice thickness was 5 mm and the interslice gap 0.5 mm in all image series of the brain.

Table S1. Technical data of the MRI of the brain.

	TR (ms)	TE (ms)	TI (ms)	Pixel size (mm)
T2w FLAIR sagittal series	9000	109	2500	0.9 x 0.9
T2wFLAIR transverse series	9560	109	2500	0.9 x 0.9
T2w turbo SE transverse series	4000	98	n/a	0.6 x 0.4
T2w turbo SE coronal series	5590	112	n/a	0.6 x 0.4
PDw SE transverse series	2390	11	n/a	0.7 x 0.6
T1w SE transverse series	594	9.1	n/a	0.8 x 0.6
T1w SE coronal series	594	9.1	n/a	1.0 x 0.9

T2w: T2-weighted; PDw: proton density weighted; T1w: T1-weighted; FLAIR: fluid attenuated inversion recovery; SE: spin echo; TR: repetition time; TE: time to echo; TI: time for inversion; n/a: not applicable.

Spinal cord

All images of the spinal cord were acquired after the brain examination, i.e. no images of the spinal cord were obtained before contrast medium administration. Time from contrast medium injection to the first T1-weighted image series of the spinal cord was 20-25 min. T1-weighted SE and T2-weighted turbo SE sagittal series were obtained first of the cervical and then of the thoracic part of the spinal cord using a slice thickness of 4 mm, an interslice gap of 0.4 mm. Transverse slices (slice thickness/interslice gap 3.0/0.3 mm) with a T1-weighted SE sequence and a T2-weighted turbo SE sequence were taken if local abnormalities were suspected in the sagittal images.

Table S2. Technical data of the MRI of the spinal cord.

	TR (ms)	TE (ms)	Pixel size (mm)
T1w SE sagittal series	500	14	1.3 x 1.3
T2w turbo SE sagittal series	4000	98	1.3 x 1.3
T1w SE transverse series	254	10	1.0 x 0.8
T2w turbo SE transverse cervical series	3800	122	0.8 x 0.6
T2w turbo SE transverse thoracal series	5030	122	0.8 x 0.6

T1w: T1-weighted; T2w: T2-weighted; SE: spin echo; TR: repetition time; TE: time to echo.

Table S3. Stratification of ongoing treatments.

Treatments	**None**	**GLA**	**IFN**	**IVIG**	**MTX**	**NZB**
RRMS, *n*	15	5	4	3	1	2
SPMS, *n*	15	0	0	0	0	1

GLA: Glatiramer acetate; NZB: Natalizumab; IFN: Interferon beta; IVIG: Intravenous IgG; MTX: Mitoxantrone.

Table S4. A summary of the clinical, radiological and protein measurements. Four of the RRMS patients had transitioned and have therefore been excluded from the RRMS group.

CRP variable	**Control mean(SD)**	**RRMS mean(SD)**	**SPMS mean(SD)**
GFAp	204(110.2)	254(163.9)	593(824.9)
sAPP-α	665(247)	649(228)	579(218)
sAPP-β	204(62.0)	187(72.1)	183(61.6)
Ab38	1440(588)	1355(542)	1315(376)
Ab40	10687(4698)	9887(4039)	9386(2677)
Ab42	1052(385)	944(343)	875(243)
Tau	226(94.4)	244(159.1)	208(58.9)
pTau	38.8(14.5)	32(9.3)	31.8(7.8)
Am	795.1(222.6)	719(176.7)	697.8(125.2)
MCP-1	511.6(125.3)	399.8(145.3)	540.3(154.4)
YKL40	74551(24302)	112217(78238)	145205(65667)
NFL	650(908)	2394(6061)	1111(728)
MBP	0.5(0.2)	0.7(0.7)	0.7(0.2)
sCD14	67542(24649)	72814(26128)	82950(18673)
sCD27	95.9(280)	525(689)	381(403)
T1	-	7(9)	15.2(9.6)
largeT1	-	0.2(0.5)	1.9(2.9)
TotalT1	-	7.3(9.4)	17.1(11.1)
TotalGd	-	4.4(14.6)	0.3(0.8)
TotalT2	-	25.6(27.6)	34.5(16.7)
T2score	-	31(37.4)	42.4(23)
Size_ventricle	-	5.8(1.9)	10.1(3.5)
Size_spinal cord	-	28(1.9)	24.4(2.1)

FGF-2	7.4(3.4)	10.5(4.7)	8.1(3.2)
Eotaxin	9.1(7.5)	9.8(6.7)	5.3(5)
TGF-α	5.3(0.9)	5.3(1.1)	4.4(0.9)
FLT-3L	30.6(5.6)	28(6.4)	29.3(5.5)
Fractalkine	71.8(9.4)	71(9.4)	66.2(8.2)
GRO	19.9(3.3)	30.6(14.2)	26.1(7.6)
MCP-3	4.3(1.3)	5.4(2.3)	4.8(1.7)
MDC	20.3(4.7)	86.6(147)	27(8.4)
PDGF-AA	3.9(1.2)	3.9(2.1)	2.6(0.5)
PDGF-BB	2.1(1.8)	2.1(1.2)	2.4(1.6)
sCD40L	11.2(3.9)	20.5(12.1)	12.8(4.2)
IP-10	726(291)	1462(1233)	1188(508)
MIP-1-α	3.4(0.6)	4.3(3.1)	4.1(2.2)
MIP-1-β	5(1.8)	6.4(2.9)	4.9(2.5)
RANTES	1.8(0.2)	3.3(2.3)	3.1(1.8)
VEGF	16(5.9)	18.2(11)	22.3(11.6)
IL-17F	0.0026(0.0025)	0.0035(0.0022)	0.0046(0.0024)
IL-10	1(0.5)	1.1(1.3)	1(0.3)
IL-12p70	0.7(0.6)	0.8(0.6)	0.7(0.4)
IL-9	2.8(0.5)	2.6(0.9)	2.8(0.9)
IL-23	0.027(0.037)	0.046(0.033)	0.043(0.044)
IL-5	0.3(0.1)	0.5(0.5)	0.7(0.6)
IL-6	1.4(0.9)	1.1(1)	1.1(1)
TNF-α	0.5(0.6)	1.2(0.9)	1.7(0.8)
IL-28A	0.0095(0.0054)	0.0099(0.0073)	0.0136(0.0062)
Galectin-9	293(76.3)	293(66.4)	380(70.9)

Table S5. Non-default parameter values used for pre-processing in OpenMS. For all parameters not mentioned, the default values were used.

Parameter	Value
Positive ion mode	
FeatureFinderMetabo	
noise_threshold_int	2500
mass_error_ppm	3.0
trace_termination_outliers	3
min_sample_rate	0.7
min_trace_length	3.0
max_trace_length	35.0
width_filtering	auto
local_rt_range	3.0
local_mz_range	11.0
charge_upper_bound	10
enable_RT_filtering	false
isotope_filtering_model	metabolites (2% RMS)
use_smoothed_intensities	false
report_convew_hulls	true
FeatureLinkerUnlabeledQT	
ignore_charge	true
max_difference (distance_RT)	10.0
max_difference (distance_MZ)	5.0
unit	ppm
Negative ion mode	
FeatureFinderMetabo	
noise_threshold_int	2500
mass_error_ppm	3.0
trace_termination_criterion	sample_rate
trace_termination_outliers	3
min_sample_rate	0.7
min_trace_length	3.0

max_trace_length	35.0
width_filtering	auto
local_rt_range	3.0
local_mz_range	11.0
charge_upper_bound	10
enable_RT_filtering	false
use_smoothed_intensities	false
report_convew_hulls	true
FeatureLinkerUnlabeledQT	
ignore_charge	true
max_difference (distance_RT)	10.0
max_difference (distance_MZ)	5.0
unit	ppm

Table S6. The change in EDSS after the follow-up period in the SPMS patients and the stratification of the three groups of clinical degree of change.

SPMS patient	EDSS at sample collection	EDSS after follow-up	ΔEDSS	Clinical degree of change
1	6	6	0	1
2	6	6	0	1
3	3	3	0	1
4	7.5	8	0.5	2
5	5	6	1	2
6	4	5.5	1.5	2
7	5	6	1	2
8	6	8	2	3
9	4.5	7	2.5	3
10	3	6.5	3.5	3
11	3	7	4	3
12	3.5	6	2.5	3
13	6.5	8.5	2	3

Table S7. Identities of the seven most frequently chosen metabolic features from the metabolomics models. Four of the features were not successfully identified.

Code name	Identified as	Class	Identifier	m/z
X1	Not identified	-	-	416.2285
X2	Not identified	-	-	174.1238
X3	Not identified	-	-	416.1671
X4	Not identified	-	-	321.1661
X5	Indolepyruvate*	Indole	HMDB60484	221.0922
X6	5,6-dihydroxyprostaglandin F1a*	Lipid	HMDB12109	409.2230
X7	20β-dihydrocortisol**	Steroid	-	365.2329

**Supported by two identification methods

* Supported by one identification method

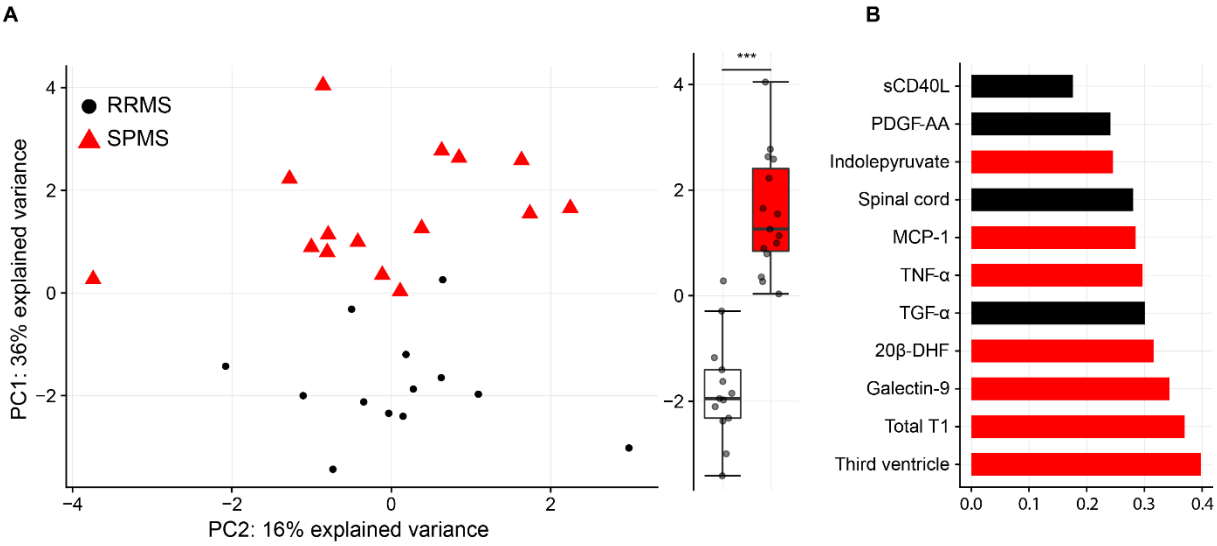

Figure S1. Principal component analysis of the CRPM dataset, excluding all patients with ongoing treatments and transitioning patients. (**A**) PCA of the eleven variables extracted by the variable selection procedure and the distribution of the first principal component to the right displaying a highly significant difference (p= 8.1e–9). (**B**) The absolute loadings for the first principal component, colored by the group with the highest mean abundance (*black*: RRMS, *red*: SPMS).

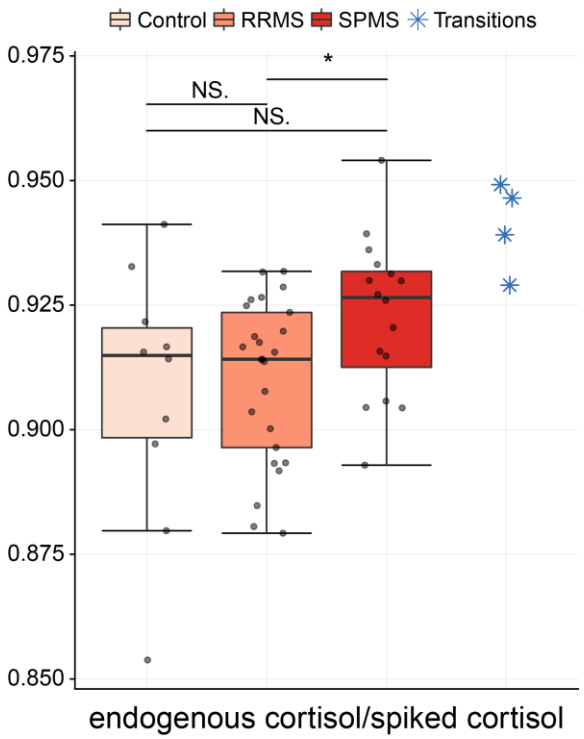

Figure S2. Endogenous cortisol levels normalized with the spiked in cortisol. Statistically significant increased levels can be seen in SPMS compared to RRMS.