Supplementary material with

Absence of B cells in brainstem and white matter lesions associates with less severe disease and absence of oligoclonal bands in MS

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Supplementary methods

Sample selection

For the immunohistochemical part of this study, three types of tissues were analyzed:

1. Standardly dissected tissue blocks at the level of the medulla oblongata (MO) were systematically examined for B cells and plasma cells. This approach allowed a standardized comparison between MS cases in the MS-autopsy cohort. The MO was selected since (1) it is one of the few regions standardly and unbiasedly dissected in the NBB-autopsy protocol, (2) it contains white matter, grey matter, and meninges and hereby covers relevant tissue compartments for MS, (3) brainstem lesions as presenting clinical symptom or on MRI scans are associated with a poor prognosis in MS patients and therefore are clinically very relevant to study.[1] MO tissue blocks were obtained from 140 MS donors, and 1 MO tissue section was analyzed per case. B-cell and plasma cell presence or absence was scored for three regions within the MO section: the meninges, the perivascular space, and the parenchyma. B cells and plasma cells were considered present when >1 individual cell could be identified in the section. The scoring was performed by an observer that was blinded for the MS-lesion characterization of these sections. MS cases with B cells in the MO (n=24) were compared to the cases without both B cells and plasma cells in the MO (n=107). MO sections that contained MS lesions and sections without any MS lesions were compared. The presence of infiltrates of B cells was scored separately when clusters of B cells were identified in the perivascular space or the meninges.[2–4]

2. In addition to the MO, to assess the consistency of findings when sampling tissue at another location, 158 subcortical white matter (WM) lesions from 73 MS cases and subcortical white matter from 24 non-neurological controls was examined for the presence of B cells and

plasma cells. The total WM per section was systematically scored, and in all positive sections, the presence of B cells and plasma cells in WM lesions was scored separately for each lesion.

3. To explore how findings in post-mortem autopsy samples of donors with advanced MS correlate with findings at the earliest stages of MS, 28 biopsies of WM lesions from 24 early MS patients were scored for the presence of B cells and plasma cells.

MS lesion characterization

Reactive, active, mixed active/inactive, inactive, and inactive remyelinated lesions were distinguished using HLA and PLP immunohistochemistry as previously described [5,6] In short, reactive sites are characterized by normal-appearing myelin with increased number of activated microglia/macrophages, active lesions show partial loss of myelin with microglia/macrophages throughout the lesion area, mixed active/inactive lesions are characterized by a demyelinated inactive center with a rim of activated microglia/macrophages, and inactive and remyelinated lesions are characterized by partial loss of myelin without any microglia/macrophages. The characterization of MS lesion subtypes in the autopsy lesions is comparable to the characterization performed in the early biopsy lesions according to Kuhlmann et al.[7]

Immunohistochemistry

Lesions were annotated, and adjacent sections were stained for CD20 (M0755, concentration 1:100; Agilent, Santa Clara, CA, USA), CD138 (MCA2459, concentration 1:500; Biorad, Hercules, CA, USA), and CD3 (GA503, concentration 1:100; Agilent) using previously described protocols.[6,8] Biopsy lesions were all characterized as active as described in Kuhlmann et al.[7] For the biopsy lesions, 4-µm thick sections were cut, and immunohistochemistry for CD20 (M0755, concentration 1:700) and CD138 (MCA2459,

concentration 1:500) was performed. Images of CD20 and CD138 immunostainings were taken using an Axioscope microscope with a micropublisher 5.0 RTV digital CCD camera (Qimaging, Surrey, BC, Canada) and the Image-Pro Plus 6.3 software (Media Cybernetics, Rockville, MD, USA). The number of T cells in MS lesions and subcortical WM were assessed using black and white images from tissue sections and particle analysis as previously described.[8] Perivascular T-cell cuffing was assessed based on CD3 immunohistochemistery, where cuffing was considered when >1 ring of $CD3^+$ cells was present, as previously described in Fransen *et al*. 2020.[8]

References

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Supplementary table 1. Donor and sample information for immunohistochemistry

Diagnosis	**Cases** (n)	**Age** (years)	**Sex**	**PMD** (h:min)	**pH value**	**Brain weight** (g)	**Disease duration** (years)
MS	140	64.8 ± 13.0	88F/52M	9:13 ± 6:40	6.5 ± 0.3	1192.0 ± 135.4	30.1 ± 13.2
RR	13	64.8 ± 16.0	8F/5M	11:52 ± 14:12	6.5 ± 0.4	1202.6 ± 102.0	25.8 ± 11.5
PP	49	67.9 ± 12.8	30F/19M	8:39 ± 4:32	6.5 ± 0.3	1193.8 ± 132.3	28.6 ± 12.3
SP	78	62.8 ± 12.3	50F/28M	9:07 ± 5:53	6.5 ± 0.3	1189.2 ± 143.0	31.8 ± 13.9
Biopsy MS donors	24	45.0 ± 13.9	18F/6M	-	-	-	-
Non-neurological controls	24	69.0 ± 12.7	13F/11M	7:53 ± 0:13	6.3 ± 0.3	1264.4 ± 142.6	-

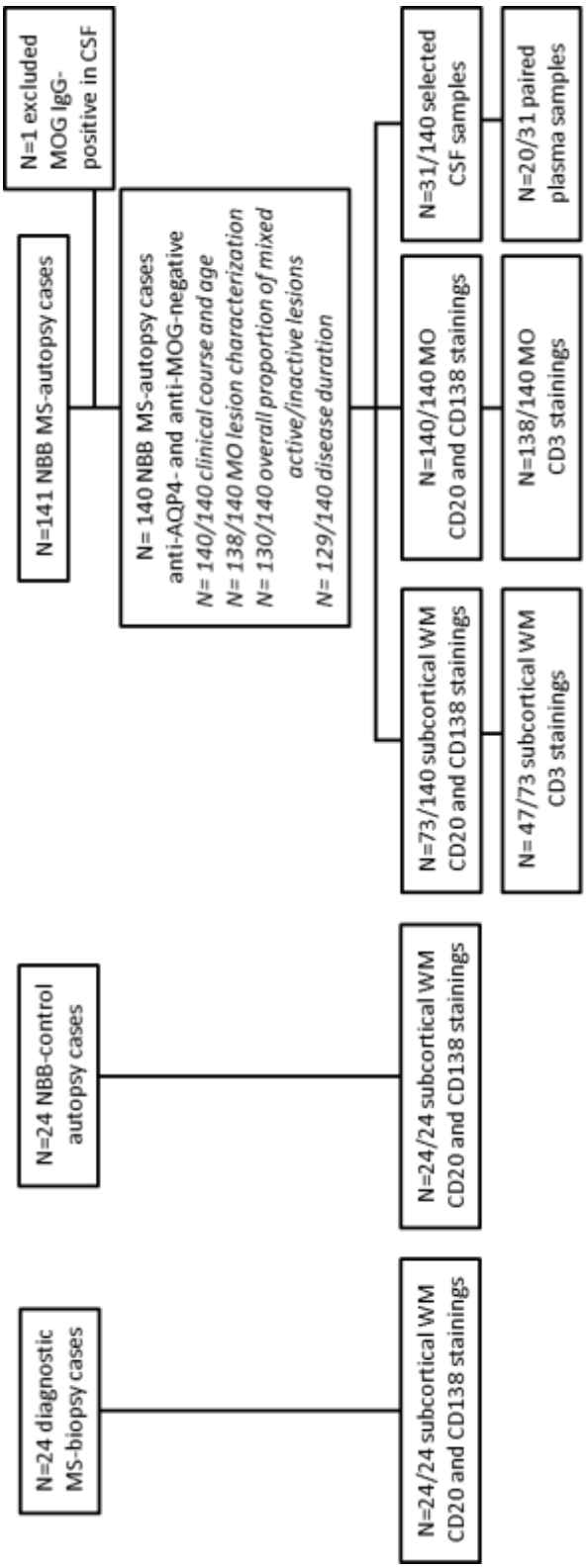
Provided is the mean ± SD (standard deviation). F, female; M, male; PMD, post-mortem delay; PP, primary progressive; RR, relapsing–remitting; SP, secondary progressive

Supplementary table 2. Disease-modifying therapy status of the MS-autopsy cohort

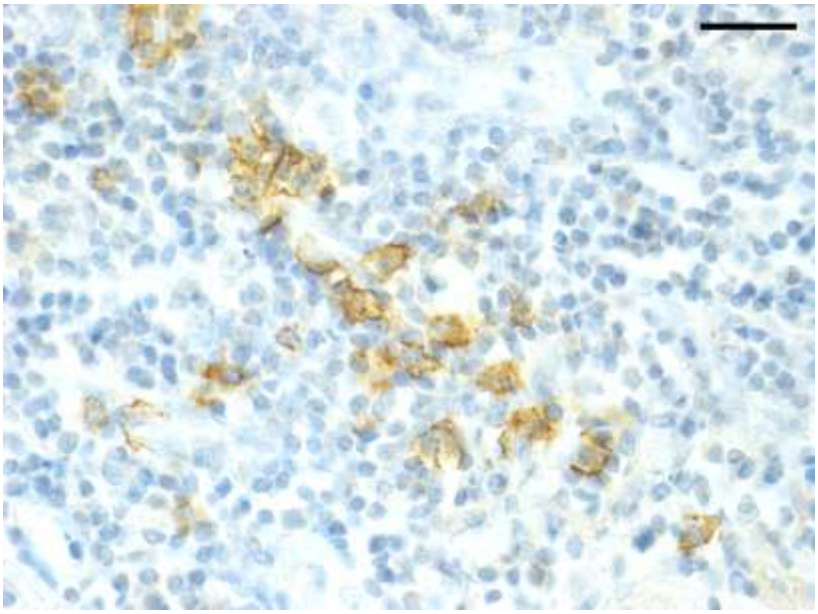
Therapy	Total (%)	B cell positive (%)	B/plasma cell negative (%)	OCB positive	OCB negative
Interferon-beta	25/136 (18)	6/21 (29)	17/106 (16)	5/25 (20)	0/6 (0)
Natalizumab	1*/136 (0)	0/21 (0)	1/106 (1)	1/25 (4)	0/6 (0)
Fingolimod	2*/136 (1)	1/21 (5)	1/106 (1)	2/25 (8)	0/6 (0)
Glatiramer acetate	3/136 (2)	0/21 (0)	2/106 (2)	1/25 (4)	0/6 (0)

Of 4 MS autopsy cases included in the study, no information on therapy status could be obtained from the clinical files. B-cell and plasma cell status was obtained at the standardized location of the MO. *One B cell-negative patient was treated with natalizumab 9 years before death and switched to fingolimod 3 years before death, with discontinuation of treatment 1 year prior to death. One B cell-positive patient received DMT (fingolimod) 1 year before death. All other patients did not receive therapy in the year before death.

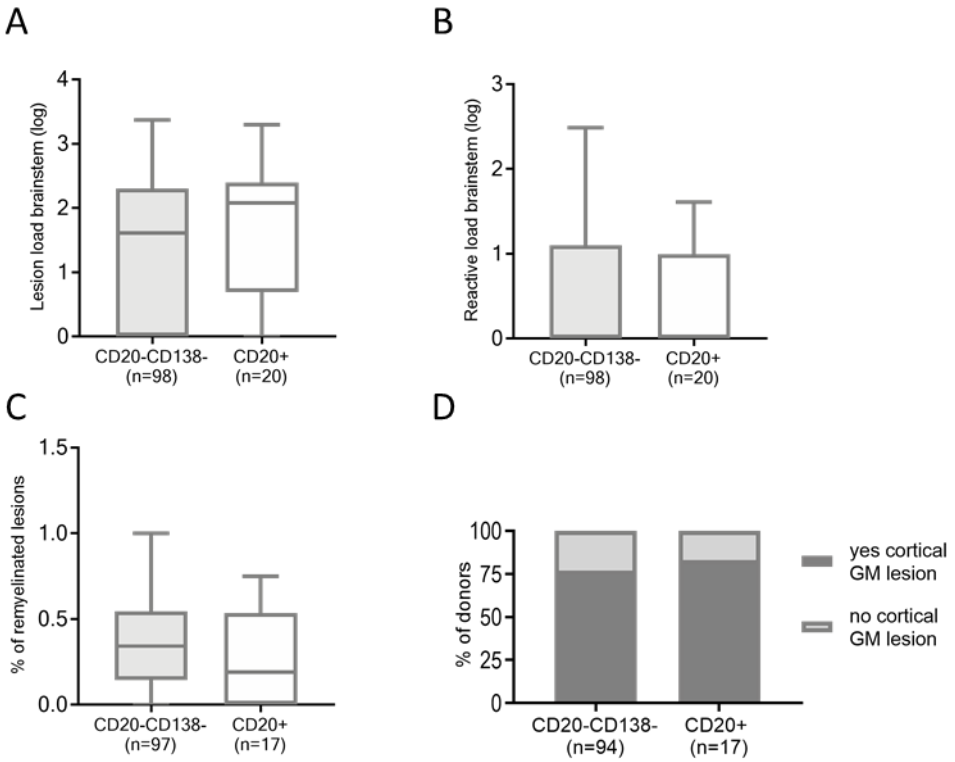
Supplementary figure 1. Flowchart of donor and sample inclusion.



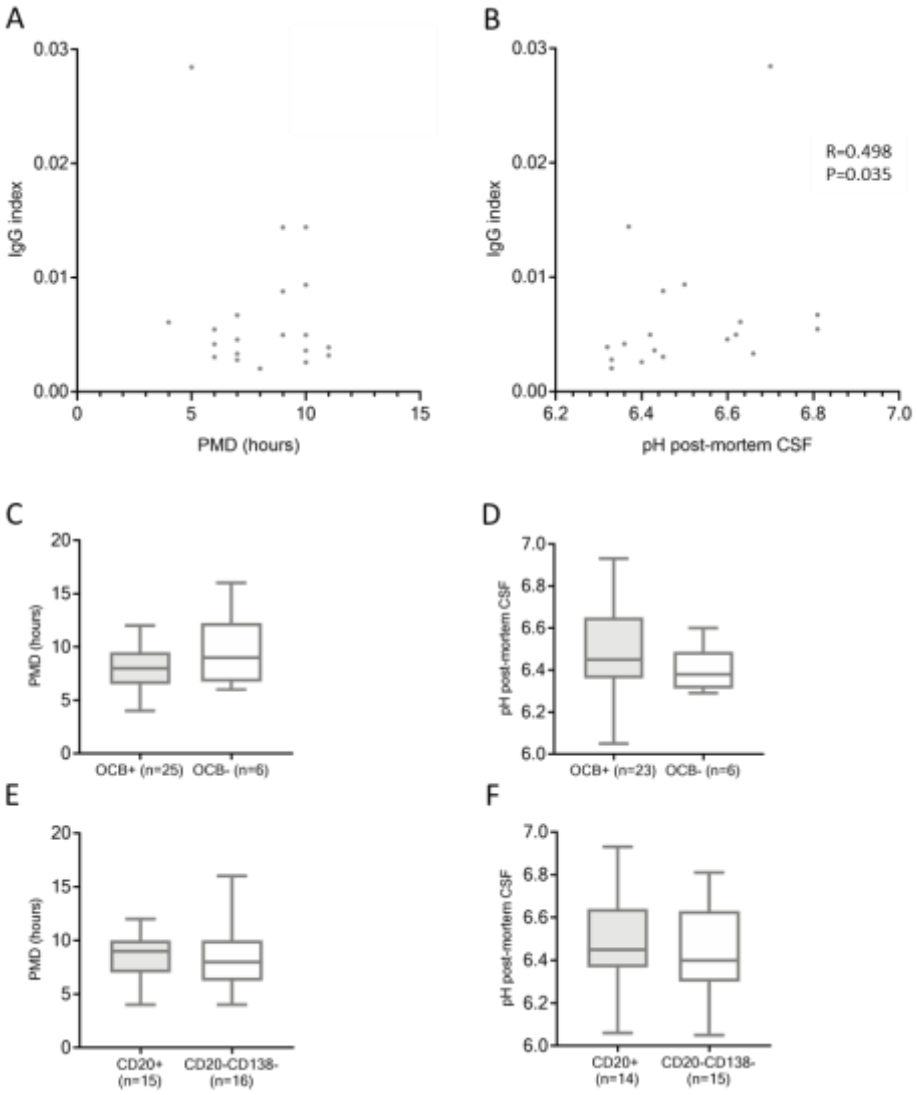
Supplementary figure 2. Control staining of tonsil tissue for $CD138^{+}$ plasma cells (scale bar is 25 μm).



Supplementary figure 3. Pathological parameters that were not significantly different between MS cases with and without B cells. (A) Number of lesions in the brainstem, (B) Number of reactive sites in the brainstem, (C) Percentage of remyelinated areas, and (D) Incidence of cortical grey matter (GM) lesions.



Supplementary figure 4. Correlation of post-mortem delay (PMD, left column) and CSF pH (right column) with (A,B) IgG index, (C,D) presence of OCB in the post-mortem CSF samples, and (E,F) presence of B cells in the MO.



Supplementary figure 5. Association of (A,B) IgG index and (C,D) OCB presence with (A,C) number of T cells in subcortical white matter and (B,D) the percentage of mixed active/inactive (mA/I) lesions.