

# 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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## Abstract

### Objective

To determine whether B-cell presence in brainstem and white matter (WM) lesions is associated with poorer pathological and clinical characteristics in advanced MS autopsy cases.

### Methods

Autopsy tissue of 140 MS and 24 control cases and biopsy tissue of 24 patients with MS were examined for CD20<sup>+</sup> B cells and CD138<sup>+</sup> plasma cells. The presence of these cells was compared with pathological and clinical characteristics. In corresponding CSF and plasma, immunoglobulin (Ig) G ratio and oligoclonal band (OCB) patterns were determined. In a clinical cohort of 73 patients, the presence of OCBs was determined during follow-up and compared to status at diagnosis.

### Results

In 34% of active and 71% of mixed active/inactive lesions, B cells were absent, which correlated with less pronounced meningeal B-cell infiltration ( $p < 0.0001$ ). The absence of B cells and plasma cells in brainstem and WM lesions was associated with a longer disease duration ( $p = 0.001$ ), less frequent secondary progressive MS compared with relapsing and primary progressive MS ( $p < 0.0001$  and  $p = 0.046$ , respectively), a lower proportion of mixed active/inactive lesions ( $p = 0.01$ ), and less often perivascular T-cell clustering ( $p < 0.0001$ ). Moreover, a lower CSF IgG ratio ( $p = 0.006$ ) and more frequent absence of OCBs ( $p < 0.0001$ ) were noted. In a clinical cohort, numbers of patients without OCBs in CSF were increased at follow-up (27.4%).

### Conclusions

The absence of B cells is associated with a favorable clinical and pathological profile. This finding may reflect extremes of a continuum of genetic or environmental constitution, but also a regression of WM humoral immunopathology in the natural course of advanced MS.

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## Glossary

**GM** = gray matter; **IgG** = immunoglobulin G; **MO** = medulla oblongata; **MOG** = myelin oligodendrocyte glycoprotein; **NBB** = Netherlands Brain Bank; **OCB** = oligoclonal band; **PP** = primary progressive; **RR** = relapsing-remitting; **SP** = secondary progressive; **T<sub>RM</sub>** = tissue-resident memory T (cells); **WM** = white matter.

MS is a heterogeneous disease differing in clinical disease course,<sup>1</sup> radiologic appearance of lesions,<sup>2</sup> and response to immunomodulatory therapies.<sup>3</sup> Of interest, variability between patients with MS is observed in the involvement of humoral immunity in the disease. At the time of diagnosis, 10% of patients with MS show the absence of oligoclonal bands (OCBs) consisting of intrathecally produced immunoglobulin (Ig) Gs.<sup>4</sup> The absence of OCBs is associated with a decreased number of lesions on MRI and a more benign disease course.<sup>4,5</sup> Furthermore, the presence of OCBs in patients with clinically isolated syndrome is associated with an increased risk for clinically definite MS and with an increased risk of disability progression.<sup>6,7</sup>

In contrast to its limited presence in early MS,<sup>8</sup> advanced progressive MS is characterized by extensive cortical demyelination.<sup>9</sup> Active cortical demyelination is observed in conjunction with the presence of meningeal follicle-like inflammatory structures.<sup>10–12</sup> The distinct zones of B cells, plasma cells, and T cells resemble tertiary lymphoid structures.<sup>12,13</sup> The presence of these follicle-like structures associates with more severe disease, reflected by an earlier disease onset and faster accumulation of disability and earlier death.<sup>11,12</sup> Recently, Reali et al.<sup>14</sup> reported that the density of meningeal B cells in MS spinal cords correlates with extensive axonal loss and white matter (WM) lesion area, but also with density of B cells in the WM perivascular space.

Besides cortical demyelination, demyelinating WM lesions also add up to disease severity in donors with advanced MS. In MS autopsy cases, the presence of active and mixed active/inactive lesions has been reported to be substantial and correlate with a short time to reaching expanded disability status scale (EDSS) end points, a shorter time to death, an unfavorable profile of risk factors for adverse outcomes, and an unfavorable profile of genetic risk factors for adverse outcomes.<sup>15,16</sup> Furthermore, we showed these active lesions to be populated by infiltrating T cells with a dominant tissue-resident memory T (T<sup>RM</sup>)-cell fraction showing signs of recent reactivation.<sup>17,18</sup> Frischer et al.<sup>19</sup> quantified the presence of B cells in MS WM lesions and found these to be predominantly present in perivascular cuffs and meninges and less frequently in the parenchyma. The presence of B cells was found most frequently in acute lesions in relapsing-remitting (RR) donors and less frequently in progressive patients.<sup>19</sup> IgG-producing cells and IgG deposits are regularly found in MS WM lesions.<sup>20,21</sup> Furthermore, the number of B cells reported in late MS autopsy lesions is highly variable between cases.<sup>19,22</sup>

The correlation of B-cell presence in WM lesions with clinical end points and risk factors as well as with meningeal B-cell infiltration has been limitedly explored. Here, we investigated the clinical and pathological characteristics of Netherlands Brain Bank (NBB) MS autopsy cases in association with B-cell infiltration of brainstem and subcortical WM lesions.

## Methods

### Donor and Sample Characteristics

One hundred forty-one MS brain donors and 24 non-neurologic controls from the NBB autopsy cohort (Amsterdam, The Netherlands) were included for this analysis of B cells and plasma cells. Donors came to autopsy between 1991 and 2015 and were diagnosed with MS according to the contemporary diagnostic criteria by their treating physicians. Clinical files were collected post-mortem by the NBB. By retrospective chart analysis, the clinical diagnosis of MS was confirmed for all patients, and the clinical course was defined as either RR, secondary progressive (SP), or primary progressive (PP) by a neurologist.<sup>15</sup> No MRI data were available. None of the donors received MS disease-modifying therapies in the year before autopsy, except for 1 (B cell–positive) donor on fingolimod. Detailed donor and tissue characteristics are described in supplementary table 1, [links.lww.com/NXI/A399](https://links.lww.com/NXI/A399), and treatment status is provided in supplementary table 2, [links.lww.com/NXI/A399](https://links.lww.com/NXI/A399). The pathological diagnosis of MS was confirmed for all cases by a certified neuropathologist.<sup>15</sup> All donors were analyzed for anti-myelin oligodendrocyte glycoprotein (MOG) and anti-aquaporin-4 antibodies using cell-based assays (figure e-1, [links.lww.com/NXI/A399](https://links.lww.com/NXI/A399)).<sup>23–25</sup>

For the immunohistochemical part of this study, 3 types of tissues were analyzed for the presence of B cells and plasma cells: (1) standardly dissected tissue blocks at the level of the medulla oblongata (MO) from 140 MS autopsy cases, (2) subcortical WM lesions from 73/140 MS autopsy cases (158 WM lesions with a median 2 lesions per donor for both the donors with and without B cells) and 24 non-neurologic control donors, and (3) early MS biopsy WM lesions (N = 28) from 24 patients with MS to explore how findings in postmortem autopsy samples of donors with advanced MS correlate with findings at the earliest stages of MS. These sections were made available by the Institute for Neuropathology, University Hospital Münster (Münster, Germany). Additional information on the analysis and selection of the different tissue samples is described in the supplementary methods, [links.lww.com/NXI/A399](https://links.lww.com/NXI/A399).

A CSF sample was acquired with a lumbar puncture from 73 patients with MS with an average disease duration of 11.7 ± 8.5 (mean ± SD) years. These patients visited the MS Center Amsterdam (Amsterdam, The Netherlands) for analysis of cognitive complaints, which is a common symptom in MS.<sup>26</sup> Information on OCB pattern at the time of diagnosis was collected by a retrospective chart analysis. Patients' characteristics are provided in table 1.

### Standard Protocol Approvals, Registrations, and Patient Consents

Informed consent was given by the donors of the NBB for brain autopsy and for the use of material and clinical data for research purposes. NBB autopsy procedures have been approved by the medical ethics committee of Amsterdam UMC, location VUmc, Amsterdam, The Netherlands.

Sampling of biopsies and CSF has been approved by the medical ethics committee of the University Hospital Münster and Amsterdam UMC, location VUmc, respectively.

### Immunohistochemistry

Immunohistochemistry of the autopsy tissue samples was performed on 8-µm-thick formalin-fixed paraffin-embedded tissue sections. All brainstem and subcortical WM tissue sections were immunostained for myelin (proteolipid protein) and HLA (HLA-DR/DQ, referred to as HLA) as previously described.<sup>15,16</sup> Lesions were annotated, and sections were stained for CD20, CD138,<sup>27</sup> and CD3 as described in the supplementary methods, links.lww.com/NXI/A399. For CD138, an image of the positive control in tonsil is provided in figure e-2, links.lww.com/NXI/A399.

**Table 1** Clinical Cohort of Patients With MS With OCB Examinations

Diagnosis	Cases (n (%))	Age at OCB (y)	Sex	MS type (%)	Disease duration at OCB (y)	Treatment (%)
<b>All MS</b>	73	49.2 ± 10.0	46F/ 27M	RR 68 SP 19 PP 5 Various 8	11.7 ± 8.5	49 DMT 51 none
<b>OCB-positive</b>	53 (72.6)	48.3 ± 9.7	32F/ 21M	RR 75 SP 19 PP 8 Various 2	11.4 ± 8.5	53 DMT 47 none
<b>OCB-negative</b>	20 (27.4)	51.5 ± 10.7	14F/6M	RR 55 SP 20 Various 25	13.2 ± 8.7	40 DMT 60 none
<b>All OCB-negative</b>						
<b>At diagnosis OCB-positive</b>	6 (30)	49.5 ± 9.7	5F/1M	BD 17 RR 67 SP 17	11.0 ± 7.0	83 DMT 17 none
<b>Previously elevated IgG, OCB unknown</b>	4 (20)	60.0 ± 9.6	2F/2M	CIS 25 RR 50 SP 25	17.3 ± 5.7	25 DMT 75 none
<b>At diagnosis OCB-negative</b>	4 (20)	49.0 ± 10.6	3F/1M	CIS 50 RR 50	6.0 ± 9.4	25 DMT 75 none
<b>Not reported</b>	6 (30)	49.5 ± 12.1	4F/2M	RR 50 SP 33 NR 17	16.0 ± 10.1	17 DMT 83 none
<b>OCB-negative, at diagnosis positive</b>						
<b>Case 1</b>		56	F	RR	20	GLA
<b>Case 2</b>		54	F	RR	8	DMF
<b>Case 3</b>		37	F	RR	13	DMF
<b>Case 4</b>		52	F	BD	4	IFNβ
<b>Case 5</b>		38	F	RR	2	DMF
<b>Case 6</b>		60	M	SP	16	None

Abbreviations: BD = Balo disease; CIS = clinically isolated syndrome; DMF = dimethyl fumarate; GLA = glatiramer acetate; IFNβ = interferon-β; IgG = immunoglobulin G; NR = not reported; OCB = oligoclonal band; PP = primary progressive; RR = relapsing-remitting; SP = secondary progressive; various = BD, CIS, and NR. Provided is the mean ± SD.

## OCB and IgG Measurement in CSF and Plasma

A selection of 16 NBB MS cases without the presence of B cells and CD138<sup>+</sup> plasma cells in the perivascular space and parenchyma of both MO and subcortical active WM lesions and 16 MS cases with B cells and CD138<sup>+</sup> plasma cells at these locations was made to analyze postmortem CSF samples. One case was excluded due to CSF anti-MOG positivity (figure e-1, [links.lww.com/NXI/A399](https://links.lww.com/NXI/A399)). Paired plasma samples were available from 20 of these MS autopsy cases (10 with B cells and 10 without B cells). In addition, paired CSF and serum samples of 73 patients with MS were analyzed. In all samples, IgG levels were determined with nephelometry, and the presence of OCBs was analyzed with isoelectric focusing followed by IgG immunoblotting.<sup>28</sup>

## Statistical Analysis

Statistical analysis was performed using GraphPad Prism 8 (8.1.1, April 2019; GraphPad, San Diego, CA, USA). Proportional differences between 2 or more strata were tested with the Fisher exact and  $\chi^2$  tests, respectively. Brainstem lesion load and reactive site load were log transformed. Normally distributed data were analyzed using a Student *t* test. A nonparametric Mann-Whitney *U* test was used when data were not normally distributed data. For disease duration and age at death a survival analysis was performed using the Gehan-Breslow-Wilcoxon test.

## Data Availability

The data that support the findings of this study are available from the corresponding author on reasonable request.

## Results

### B Cells Are Present in Early MS Biopsy Lesions and in Active and Mixed Active/Inactive MS Autopsy Lesions

Of the NBB MS autopsy cohort, we analyzed the material of *N* = 140 donors for the current study. First, we analyzed the presence of B cells and CD138<sup>+</sup> plasma cells in the MO because this is one of the few standardly dissected regions in the NBB MS autopsy protocol, which contains both the WM and gray matter (GM) brain parenchyma and the meninges. B cells were more often present in the perivascular space (*p* = 0.004) and meninges (*p* < 0.0001) compared with the brain parenchyma (17%, 51%, and 4% of cases, respectively; figure 1, A and B). In the MO collection, 85 sections contained MS lesions, and 53 sections did not contain MS lesions. B cells and CD138<sup>+</sup> plasma cells were found more frequently (*p* = 0.028 and *p* = 0.038, respectively) in sections with MS lesions (21% had B cells and 19% had plasma cells) compared with normal-appearing MO tissue sections (9% had B cells, and 8% had plasma cells) (figure 1, C and D).

To investigate the association with WM lesion characteristics, we scored the presence of B cells and CD138<sup>+</sup> plasma cells in subcortical WM from 24 non-neurologic controls and 73 MS

autopsy cases, containing 158 MS lesions (10 reactive, 41 active, 66 mixed active/inactive, 25 inactive, and 16 remyelinated). Moreover, we determined the presence of B cells and CD138<sup>+</sup> plasma cells in 28 early MS biopsies from WM lesions, which were all active. In the non-neurologic controls, we identified B cells (2–5 cells per section) in the meninges in 4% (2 of 24) of the cases. B cells were more frequently found in early biopsy (93%; *p* < 0.0001) and in active (66%; *p* < 0.0001) and mixed active/inactive (29%; *p* < 0.0001) autopsy MS lesions compared with control WM. Notably, in 34% of the active autopsy lesions, no B cells were identified. In reactive (10%), inactive (8%), and remyelinated lesions (6%), B cells were not significantly enriched compared with control WM (figure 1E). In control WM and meninges, no CD138<sup>+</sup> plasma cells were identified. CD138<sup>+</sup> plasma cells were found in early biopsy lesions (56%; *p* = 0.004) and in all autopsy MS lesion subtypes—reactive (10%; *p* = 0.002), active (22%; *p* < 0.0001), mixed active/inactive (8%; *p* = 0.007), inactive (24%; *p* < 0.0001), and remyelinated (19%; *p* < 0.0001) compared with control WM (figure 1, F–I).

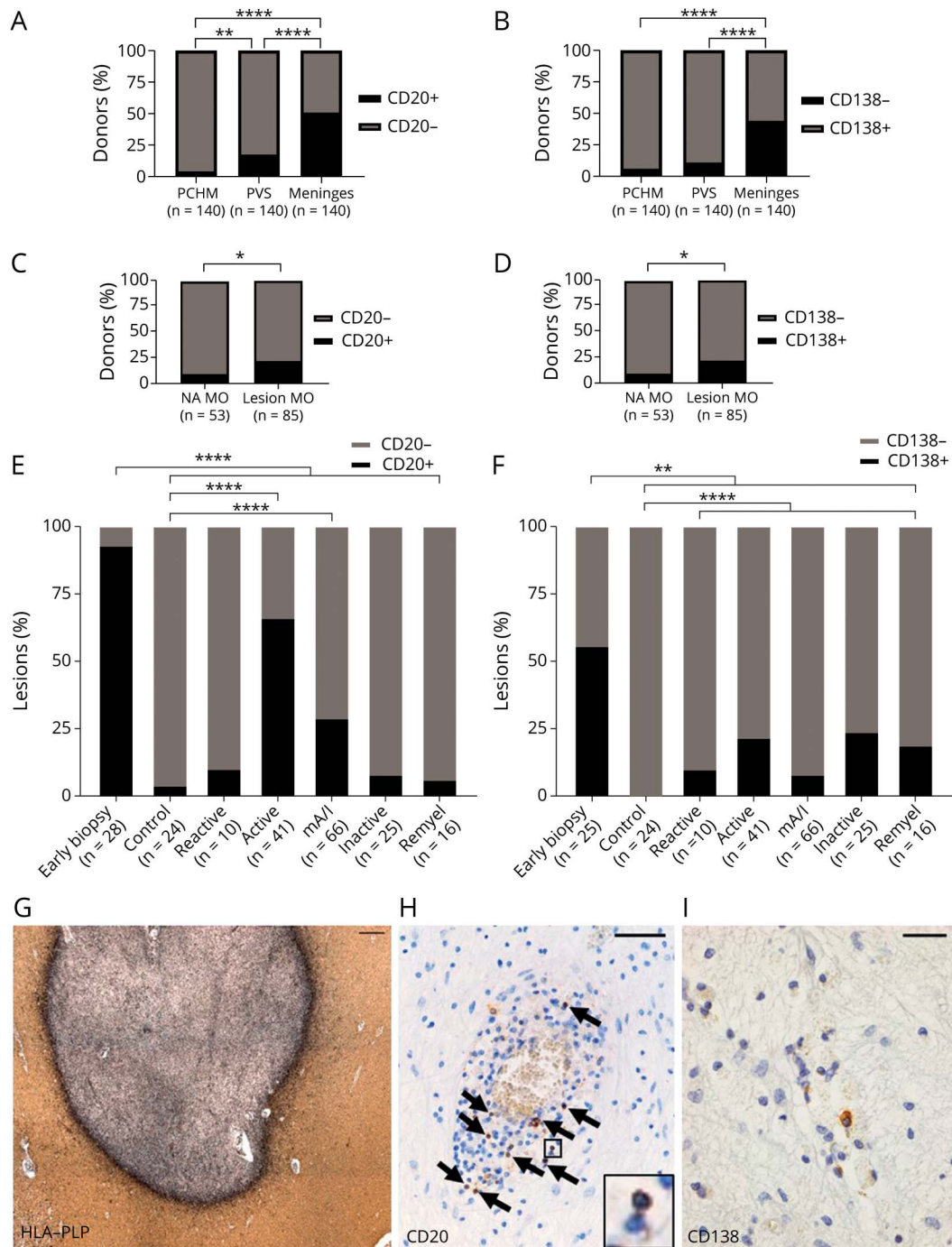
### The Presence of B Cells and CD138<sup>+</sup> Plasma Cells Is a General Donor Characteristic

We next assessed the presence of B cells and CD138<sup>+</sup> plasma cells within multiple locations (parenchyma, perivascular space, and meninges) and tissue blocks (MO and subcortical WM) from the same donors (figure 2, A and B). The presence of B cells in the perivascular space was associated with the presence of B cells in the meninges (92% and 43% in donors with and without perivascular B cells, respectively; *p* < 0.0001; figure 2D). This is in accordance with Reali et al.,<sup>14</sup> who also observed an increased number of B cells in meninges to be associated with an increased number of B cells in perivascular space of MS spinal cords. Furthermore, the presence of B cells in the MO was associated with the presence of B cells in the subcortical WM (50% and 27% in donors with and without MO B cells, respectively; *p* = 0.001; figure 2E).

### Limited Presence of B Cells in MS Autopsy Cases Associates With a Favorable Clinical and Pathological Profile

To assess whether the presence of B and CD138<sup>+</sup> plasma cells in MO and subcortical WM lesions correlates with more severe MS, likewise earlier reported for meningeal B-cell infiltrates, we compared donors with and without B cells at these locations. B cells were frequently encountered in perivascular clusters with T cells (figure 3A). Cases without B cells at the MO showed less often perivascular cuffing of T cells in the MO (11% and 35%; *p* < 0.0001, figure 3B). Cases without B cells in subcortical WM showed a trend for a lower number of T cells in subcortical MS lesions (median 3.3 vs 8.3 cells/mm<sup>2</sup>; *p* = 0.06; figure 3C) and a lower overall percentage of mixed active/inactive lesions (mean 23.7% vs 39.6%; *p* = 0.01; figure 3D) compared with MS donors with B cells. Clinically, they showed a higher age at death (median 69.0 vs 55.5; *p* = 0.0006; figure 3E) and a less severe clinical disease course, defined as a longer disease duration (median 31.0 vs 22.0; *p* =

**Figure 1** B Cells Are Enriched in Biopsy Lesions and Active and Mixed Active/Inactive Lesions at Autopsy



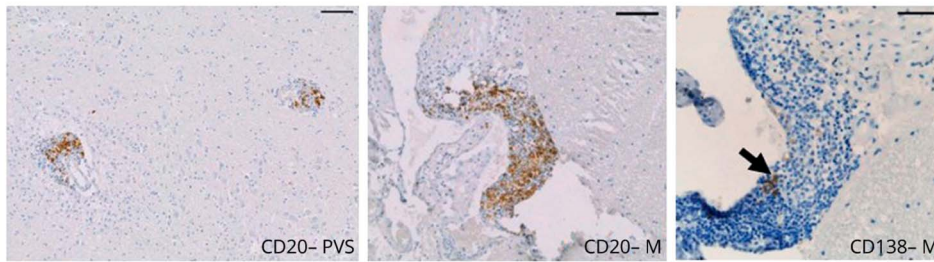
(A/B) B cells and plasma cells were enriched in the perivascular space and meninges of the MO compared with the MO parenchyma. (C/D) MO lesions contained more frequently B cells and plasma cells compared with the normal-appearing medulla oblongata (NA MO). MS lesion subtypes were analyzed in the subcortical WM. (E) Early MS biopsy lesions significantly more often contained B cells compared with all autopsy lesions. In active and mixed active/inactive autopsy lesions, B cells were significantly more often present compared with control WM. (F) Early MS biopsy lesions significantly more often contained plasma cells compared with autopsy lesions. Plasma cells were significantly more often present in all MS lesion types compared with control WM. (G) Example of an inflammatory active MS lesions of a secondary progressive MS brain donor with MS for 27 years, stained for HLA (black) and proteolipid protein (brown). Scale bar is 500  $\mu$ m. (H/I) In the perivascular space, B cells (CD20<sup>+</sup>, panel H, scale bar is 50  $\mu$ m) and a plasma cell (CD138<sup>+</sup>, panel I, scale bar is 25  $\mu$ m) were present (both brown color). \* $p$  < 0.05, \*\* $p$  < 0.01, and \*\*\*\* $p$  < 0.0001. WM = white matter

0.007; figure 3F), and they more often had a persistent relapsing or PP course compared with an SP course (100% and 87% vs 75%;  $p$  < 0.0001 and  $p$  = 0.046; figure 3G). There was no difference in brainstem lesion load, reactive site load,

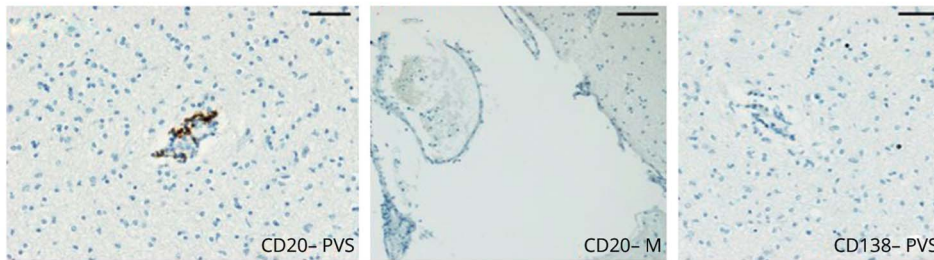
percentage of inactive remyelinated areas, and incidence of cortical GM lesions between MS cases with and without B cells at the investigated locations (figure e-3, links.lww.com/NXI/A399).

**Figure 2** B-Cell and Plasma Cell Presence in the Meninges and Perivascular Space Is Consistent Within Donors

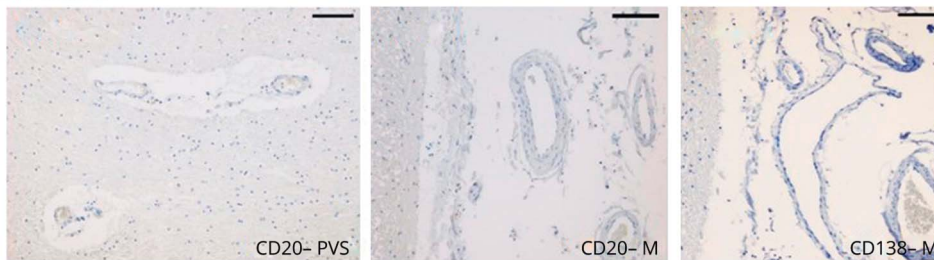
**A. Donor 1**



**B. Donor 2**

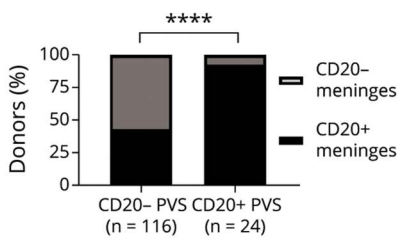


**C. Donor 3**

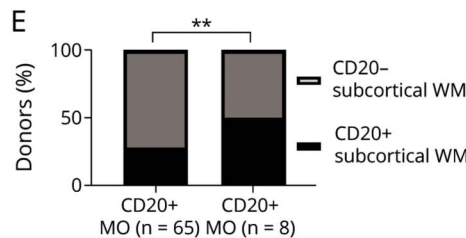


(A) In the MO of donor 1 with 27 years of secondary progressive MS, B-cell and plasma cell infiltrates were identified in both the perivascular space (PVS) and the meninges (M). Scale bars are 100  $\mu$ m for CD20 and 50  $\mu$ m for CD138. (B) In the MO of donor 2 with a primary progressive disease course and a disease duration of 2 years, B cells were detected in the perivascular space but not in the meninges, and no plasma cells were identified. Scale bars are 50  $\mu$ m for CD20 and CD138 in the PVS and 100  $\mu$ m for CD20 in the meninges. (C) In the MO of donor 3 with a relapsing disease course for 38 years, no B cells or plasma cells were identified in both the meninges and the perivascular space. Scale bars are as in A. (D) The absence of B cells in the perivascular space (PVS) is associated with the absence of B cells in the meninges. (E) The absence of B cells in the MO is associated with the absence of B cells in the subcortical white matter. \*\* $p < 0.01$  and \*\*\*\* $p < 0.0001$ . MO = medulla oblongata

**D**



**E**



**MS Autopsy Cases With Limited Presence of B Cells Show a Lower Intrathecal IgG Production and Lack More Often OCBs**

Because our data suggest an association between the presence of B cells in meninges and MO/subcortical WM, as well as an association with a more severe pathological and clinical profile, we explored its relevance for intrathecal B-cell activation. Because an increased intrathecal IgG production and OCB presence are highly correlating biomarkers of MS,<sup>29</sup> and the presence of OCBs is associated with adverse outcomes,<sup>6,30</sup> we explored whether these CSF biomarkers were associated with B-cell and CD138<sup>+</sup> plasma-cell presence in MO and subcortical WM lesions. We conducted an extreme-of-outcome analysis by selecting 16 cases with B cells and 16 cases without B cells and CD138<sup>+</sup> plasma cells in both MO and subcortical WM

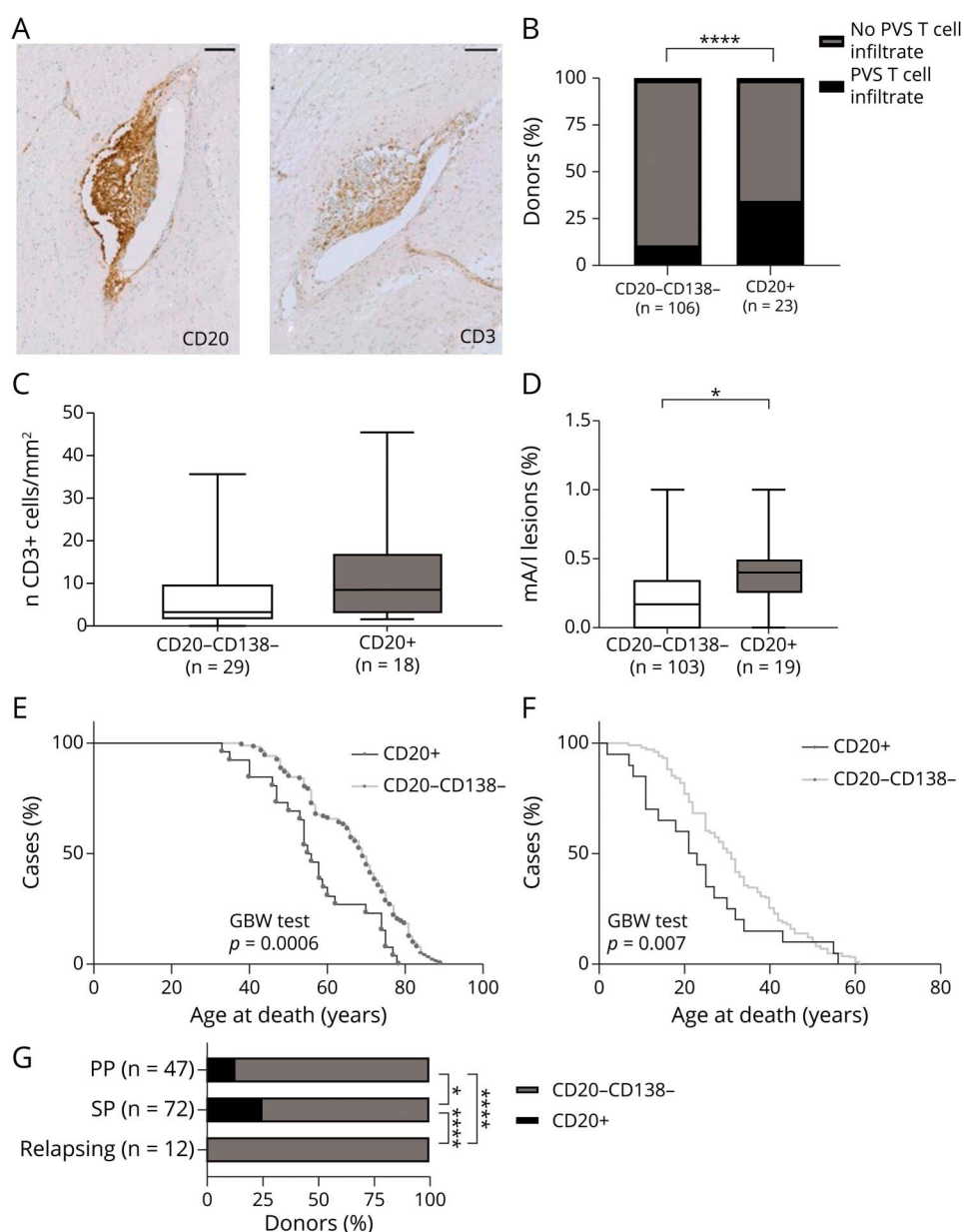
lesions. One MS case with B cells and CD138<sup>+</sup> plasma cells was excluded before OCB analysis because anti-MOG antibodies were detected in the postmortem CSF. There was no significant association of IgG index and OCB presence (figure 4A) with postmortem delay. The pH of postmortem CSF showed a positive correlation with IgG index (Spearman correlation  $R = 0.498$ ;  $p = 0.035$ ), but not with OCB presence or presence of B cells (figure e-4, links.lww.com/NXI/A399). Selected cases lacking B cells and CD138<sup>+</sup> plasma cells in MO and subcortical WM lesions showed a lower CSF IgG level (median 0.04 vs 0.07;  $p = 0.02$ , figure 4B) and a lower IgG CSF/plasma ratio (median 0.003 vs 0.008;  $p = 0.007$ , figure 4C), indicating a lower intrathecal IgG production compared with MS cases with B cells. CSF OCBs were absent in 37% of cases without B cells and CD138<sup>+</sup> plasma cells, whereas all cases with

B cells displayed CSF OCBs ( $p < 0.0001$ ; figure 4D). This observation suggests that these MS cases with limited presence of B cells and CD138<sup>+</sup> plasma cells in MO and subcortical WM lesions are characterized by an overall altered CSF IgG clonality and lower IgG production. This observation is again in line with the strong correlation reported by Reali et al.<sup>14</sup> between meningeal and perivascular B-cell presence. In addition, the IgG index, but not the presence of OCBs, was positively correlated with the number of T cells in subcortical WM (figure e-5A, links.lww.com/NXI/A399). Other pathological end points did not correlate with IgG production or clonality (figure e-5B–D, links.lww.com/NXI/A399).

### OCBs Can Disappear Over Time in Patients With MS

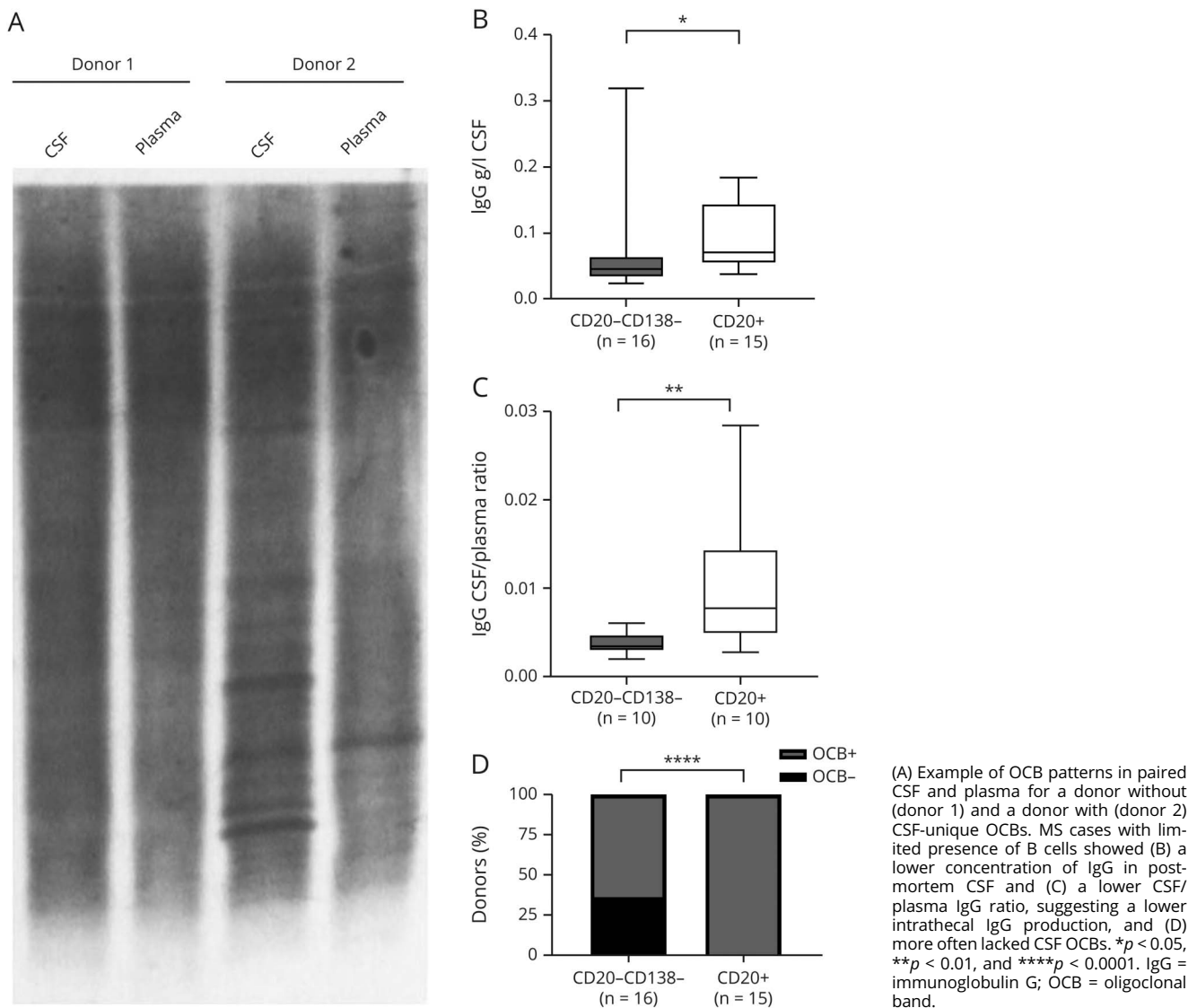
In MS autopsy cases selected for absence of B cells and CD138<sup>+</sup> plasma cells, the prevalence of OCBs was lower than the expected 90% OCB positivity of patients with MS at diagnosis.<sup>31</sup> This difference could be explained by selection of MS donors with an extreme profile of genetic or environmental factors,<sup>16</sup> but also by a decline of the intrathecal humoral immune response over time in chronic MS. In our current study, 2 of 6 MS cases without OCBs at autopsy had an elevated IgG ratio at diagnosis without information on OCBs, 1 of 6 donors had normal diagnostic CSF examination, and no information was available for the 3 other donors. To explore whether a dynamic course of

**Figure 3** MS Cases With Limited Presence of B Cells Show a Favorable pathological and Clinical Profile



(A) B cells were often encountered in perivascular clusters together with T cells. Scale bars are 100 μm. (B) MS cases with the limited presence of B cells showed less often perivascular clustering of CD3<sup>+</sup> T cells, (C) a lower number of CD3<sup>+</sup> T cells in MS lesions, (D) a lower percentage of mixed active/inactive (mAI) lesions, (E) a higher age at death, (F) a longer disease duration, and (G) more often a secondary progressive disease course. \* $p < 0.05$  and \*\*\*\* $p < 0.0001$ .

**Figure 4** MS Cases With Limited B Cells Show Lower Intrathecal IgG Production and More Often Lack OCBs



OCB pattern throughout the disease course of MS can be a plausible explanation of our findings, the presence of OCBs was determined in a clinical cohort of 73 patients with MS who underwent a lumbar puncture after an average disease duration of  $11.7 \pm 8.5$  (mean  $\pm$  SD) years. In 27.4% (20 of 73) of the patients with MS, OCBs were absent. In 6 of the 20 patients with MS without OCBs at follow-up, OCBs were present at the time of diagnosis (table 1). Although laboratory differences can be confounders, these data support the hypothesis that the contribution of B cells to MS pathology may decline during the course of MS.

## Discussion

We here demonstrate the absence of B cells and CD138<sup>+</sup> plasma cells in 34% of the active WM lesions of advanced

MS cases in the NBB autopsy cohort. Cases without B cells at the MO or subcortical WM showed a more favorable pathological profile as indicated by a lower number of T cells in MS lesions, a lesser frequency of perivascular cuffing of T cells, and a lower percentage of mixed active/inactive lesions. Clinically, they manifested with a less frequent SP disease course, a longer disease duration, and a lower percentage of mixed active/inactive lesions compared with the MS donors with B cells in MO or subcortical WM lesions. Furthermore, a selected subgroup of patients with MS without WM B cells and CD138<sup>+</sup> plasma cells had a lower intrathecal IgG production and lacked more often unique OCBs in postmortem CSF. Our findings indicate that besides an important role of meningeal B cells in cortical pathology of advanced progressive MS, B-cell infiltration in WM is also a detrimental phenomenon at the later stages of MS.



In MS and also other autoimmune diseases, B cells have been described to play an important role in antigen presentation and cytokine production, which induces the activation and proliferation of T cells.<sup>32–35</sup> MS cases with B cells show an increased number of T cells in their MS lesions suggesting increased T-cell activation. We and others previously showed that reactivated T<sub>RM</sub> cells are associated with the ongoing inflammatory lesion activity in WM lesions from advanced MS cases.<sup>19,22,36</sup> In MS lesions, these reactivated T<sub>RM</sub> cells are often encountered in clusters in the perivascular space together with B cells,<sup>17,37</sup> suggesting that antigen presentation and reactivation of T<sub>RM</sub> cells induced by B cells potentially occur at this location.<sup>17,18,38</sup> This illustrates that besides IgG production, B cells may have different functional roles in MS WM lesions.

We show that CD138<sup>+</sup> plasma cells are present more often in MS lesions compared with control and normal-appearing WM in line with earlier reports, however, only in a low percentage of the MS autopsy cases.<sup>20,21</sup> Of interest, CD138<sup>+</sup> plasma cells were most often present in inactive lesions compared with the other lesion subtypes. Prineas et al.<sup>21</sup> previously showed in a detailed electron microscopy study of the perivascular space in MS tissues that high numbers of plasma cells are present in inactive lesion areas. This suggests that CD138<sup>+</sup> plasma cells play a less prominent role compared with B cells in the ongoing microglial activity of MS lesions. Ocrelizumab and rituximab, which show an effect on disease progression in MS, are directed against circulating CD20<sup>+</sup> B cells but do not affect CD138<sup>+</sup> plasma cells.

A large heterogeneity in the number of B cells and the presence of IgG depositions in MS lesions has been described over the past decades. In both early MS biopsies and late MS autopsies, the presence<sup>39–41</sup> and absence<sup>39,42</sup> of IgG deposits in MS lesions have been described. Also, the number of B cells in MS autopsy lesions is highly heterogeneous between MS cases. In 34% of the inflammatory active MS lesions in autopsy tissue, we identified no B cells, and we showed that the presence of B cells correlates between different location (MO and subcortical WM) and compartments (parenchyma, perivascular space, and meninges) in an individual donor.

In a selected subgroup of MS cases without WM B cells, a lower intrathecal IgG production and a more frequent absence of OCBs were found. The presence of OCBs in 60% in these MS cases is lower compared with clinical MS cohorts, where 90% showed OCBs at diagnosis.<sup>4</sup> Possibly, we now selected an extreme subgroup of MS cases with a genetic profile at one side of a continuum that restricts involvement of B cells in MS lesion pathogenesis.<sup>16</sup> Alternatively, because we identified B cells in 92% of the early MS biopsy lesions, and the MS cases with limited B-cell presence in autopsy tissue had a longer disease duration and older age, B-cell involvement in WM lesion activity might be extinguishing over time. Accordingly, Frischer et al.<sup>19</sup> reported higher numbers of perivascular B cells in donors with relapsing and

progressive disease compared with inactive disease. We provided some support for this hypothesis, by observing in a clinical cohort the absence of OCBs in 27.4% of patients after a disease duration of  $11.7 \pm 8.5$  (mean  $\pm$  SD) years. In 6 of these patients without OCBs, their presence at diagnosis could be validated. In 4 of these patients, the elevated IgG index at diagnosis was validated. These data require careful interpretation because comparison with historical data on OCB presence may be inaccurate. It is not likely that treatment with disease-modifying therapies confounds these results. In clinical studies, the presence of CSF OCBs was not affected by highly efficacious therapies as fingolimod,<sup>43</sup> rituximab,<sup>44</sup> and alemtuzumab,<sup>45</sup> whereas treatment with natalizumab<sup>46,47</sup> and cladribine<sup>48</sup> was associated with reduced OCBs. Treatment with dimethyl fumarate has not been associated with lower CSF IgG production.<sup>49</sup> Although loss of CSF OCBs has been described in a cohort of interferon-beta- and glatiramer acetate-treated patients with MS,<sup>50</sup> this has not been observed in controlled studies. Whether the absence of perivascular B cells truly is a biomarker for the regression of WM inflammatory disease activity in advanced MS remains to be determined. Regarding cessation of disease-modifying therapies in advanced MS, this could be a clinically useful hypothesis to pursue.

Our study has some limitations. Because of the structure of the NBB MS tissue dissection protocol, we could only investigate the presence of B cells at selected locations in WM and meninges. Bias in our data by sample and site selection cannot be excluded, which may be partially overcome by selecting a standardly dissected location and comparing multiple locations within the same donor. The extreme outcome analysis, comparing donors with or without B cells at multiple locations in a dichotomous approach, provides a rather crude estimate of biological associations than correlation analyses. However, due to limited availability of material, this was for the current research question in this cohort the most feasible approach.

In sum, we here demonstrate in an advanced MS autopsy cohort that the absence of B cells at the MO and subcortical WM is associated with a favorable clinical and pathological profile. This finding may reflect extremes of a continuum of genetic or environmental constitution but also a regression of WM humoral immunopathology in the natural course of advanced MS.

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## Appendix (continued)

Name	Location	Contribution
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## 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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