ARTICLE OPEN ACCESS

Absence of B Cells in Brainstem and White Matter Lesions Associates With Less Severe Disease and Absence of Oligoclonal Bands in MS

Nina L. Fransen, MD, Brigit A. de Jong, MD, PhD, Katharina Heß, MD, Tanja Kuhlmann, MD, PhD, Maria C.J. Vincenten, MSc, Jörg Hamann, PhD, Inge Huitinga, PhD,* and Joost Smolders, MD, PhD*

Correspondence
Dr. Smolders
j.j.f.m.smolders@erasmusmc.nl

Neurol Neuroimmunol Neuroinflamm 2021;8:e955. doi:10.1212/NXI.0000000000000955

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 $CD20^+$ B cells and $CD138^+$ 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.

*Co–senior authors.

From the Department of Neuroimmunology (N.L.F., M.C.J.V. J.H., I.H., J.S.), Netherlands Institute for Neuroscience, Amsterdam, The Netherlands; Department of Neurology and MS Center, Amsterdam, Amsterdam Neuroscience, Amsterdam University Medical Centers, Vrije Universiteit (B.A.J.), The Netherlands; Institute for Neuropathology (K.H., T.K.), University Hospital Münster, Münster, Germany; Department of Experimental Immunology (J.H.), Amsterdam Infection & Immunity Institute, Amsterdam University Medical Centers, University of Amsterdam, The Netherlands; Swammerdam Institute for Life Sciences, University of Amsterdam (I.H.), The Netherlands; and MS Center ErasMS (J.S.), Departments of Neurology and Immunology, Erasmus Medical Center, Rotterdam, The Netherlands.

Go to Neurology.org/NN for full disclosures. Funding information is provided at the end of the article.

The Article Processing Charge was funded by the authors.

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; $\mathbf{T_{RM}}$ = tissue-resident memory T (cells); **WM** = white matter.

MS is a heterogeneous disease differing in clinical disease course,[1] radiologic appearance of lesions,[2] and response to immunomodulatory therapies.[3] 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.[4] The absence of OCBs is associated with a decreased number of lesions on MRI and a more benign disease course.[4,5] 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.[6,7]

In contrast to its limited presence in early MS,[8] advanced progressive MS is characterized by extensive cortical demyelination.[9] Active cortical demyelination is observed in conjunction with the presence of meningeal follicle-like inflammatory structures.[10–12] The distinct zones of B cells, plasma cells, and T cells resemble tertiary lymphoid structures.[12,13] 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.[11,12] Recently, Reali et al.[14] 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.[15,16] Furthermore, we showed these active lesions to be populated by infiltrating T cells with a dominant tissue-resident memory T (T^{RM})-cell fraction showing signs of recent reactivation.[17,18] Frischer et al.[19] 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.[19] IgG-producing cells and IgG deposits are regularly found in MS WM lesions.[20,21] Furthermore, the number of B cells reported in late MS autopsy lesions is highly variable between cases.[19,22]

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 postmortem 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.[15] 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, and treatment status is provided in supplementary table 2, links.lww.com/NXI/A399. The pathological diagnosis of MS was confirmed for all cases by a certified neuropathologist.[15] 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).[23–25]

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.

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.[26] 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.[15,16] Lesions were annotated, and sections were stained for CD20, CD138,[27] 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 (%)
All MS	73	49.2 ± 10.0	46F/ 27M	RR 68 SP 19 PP 5 Various 8	11.7 ± 8.5	49 DMT 51 none
OCB-positive	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
OCB-negative	20 (27.4)	51.5 ± 10.7	14F/6M	RR 55 SP 20 Various 25	13.2 ± 8.7	40 DMT 60 none
All OCB-negative						
At diagnosis OCB-positive	6 (30)	49.5 ± 9.7	5F/1M	BD 17 RR 67 SP 17	11.0 ± 7.0	83 DMT 17 none
Previously elevated IgG, OCB unknown	4 (20)	60.0 ± 9.6	2F/2M	CIS 25 RR 50 SP 25	17.3 ± 5.7	25 DMT 75 none
At diagnosis OCB-negative	4 (20)	49.0 ± 10.6	3F/1M	CIS 50 RR 50	6.0 ± 9.4	25 DMT 75 none
Not reported	6 (30)	49.5 ± 12.1	4F/2M	RR 50 SP 33 NR 17	16.0 ± 10.1	17 DMT 83 none
OCB-negative, at diagnosis positive						
Case 1		56	F	RR	20	GLA
Case 2		54	F	RR	8	DMF
Case 3		37	F	RR	13	DMF
Case 4		52	F	BD	4	IFNβ
Case 5		38	F	RR	2	DMF
Case 6		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$^+$ 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$^+$ 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). 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.[28]

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 χ^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$^+$ 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$^+$ 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$^+$ 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$^+$ 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$^+$ plasma cells were identified. CD138$^+$ 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).

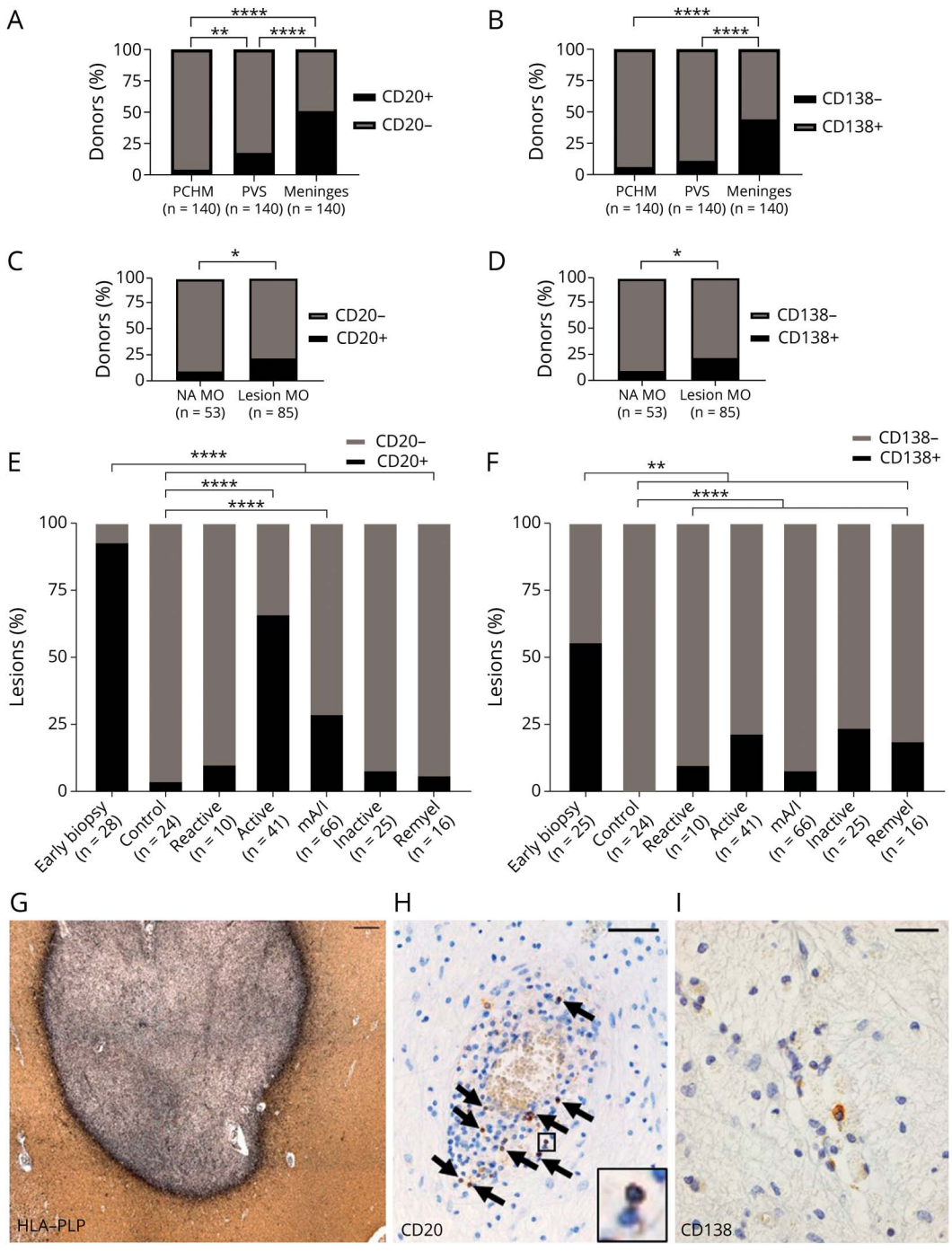
The Presence of B Cells and CD138$^+$ Plasma Cells Is a General Donor Characteristic

We next assessed the presence of B cells and CD138$^+$ 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.,[14] 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$^+$ 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^2; 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 =

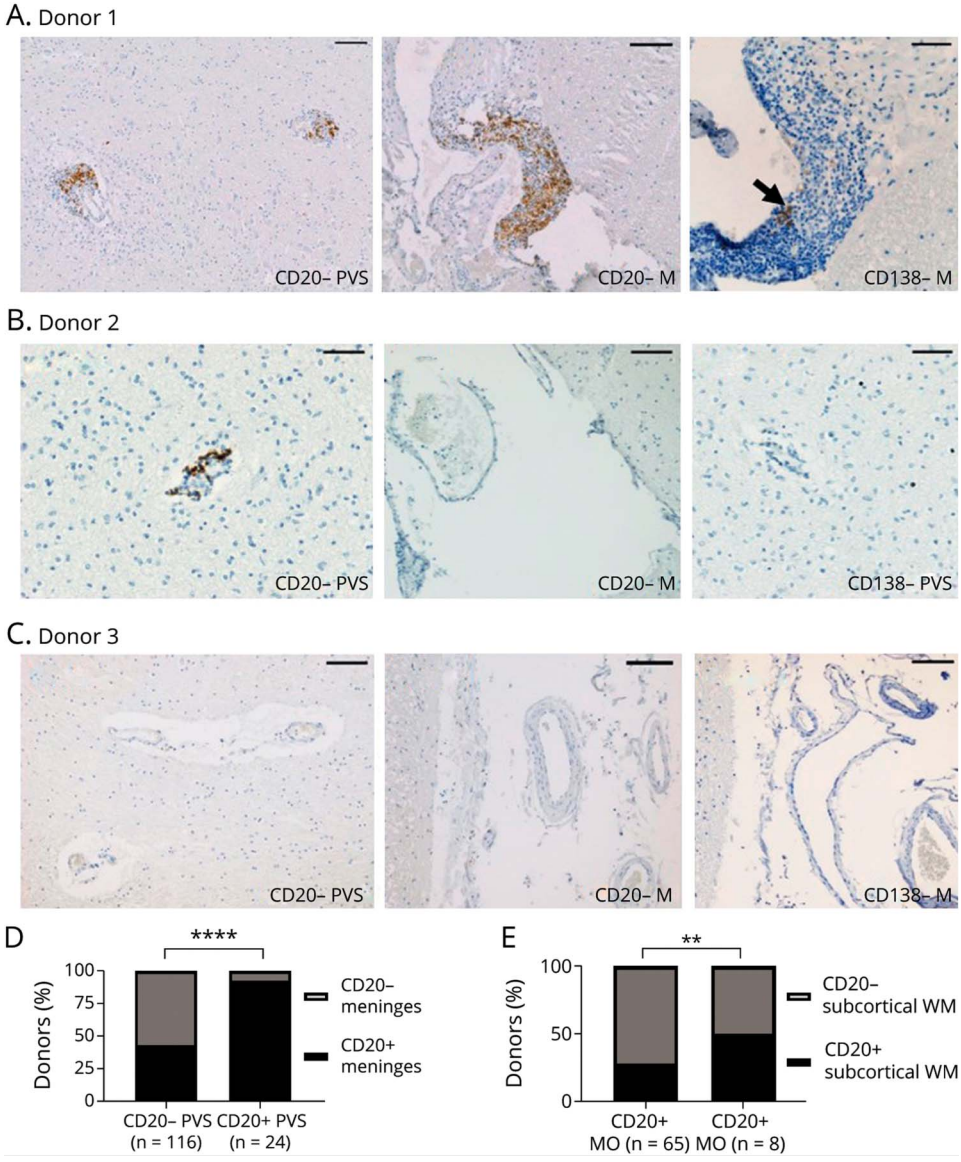
Figure 1 B Cells Are Enriched in Biopsy Lesions and Active and Mixed Active/Inactive Lesions at Autopsy



(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 μm. (H/I) In the perivascular space, B cells ($CD20^+$, panel H, scale bar is 50 μm) and a plasma cell ($CD138^+$, panel I, scale bar is 25 μm) 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) 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 μm for CD20 and 50 μm 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 μm for CD20 and CD138 in the PVS and 100 μm 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

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

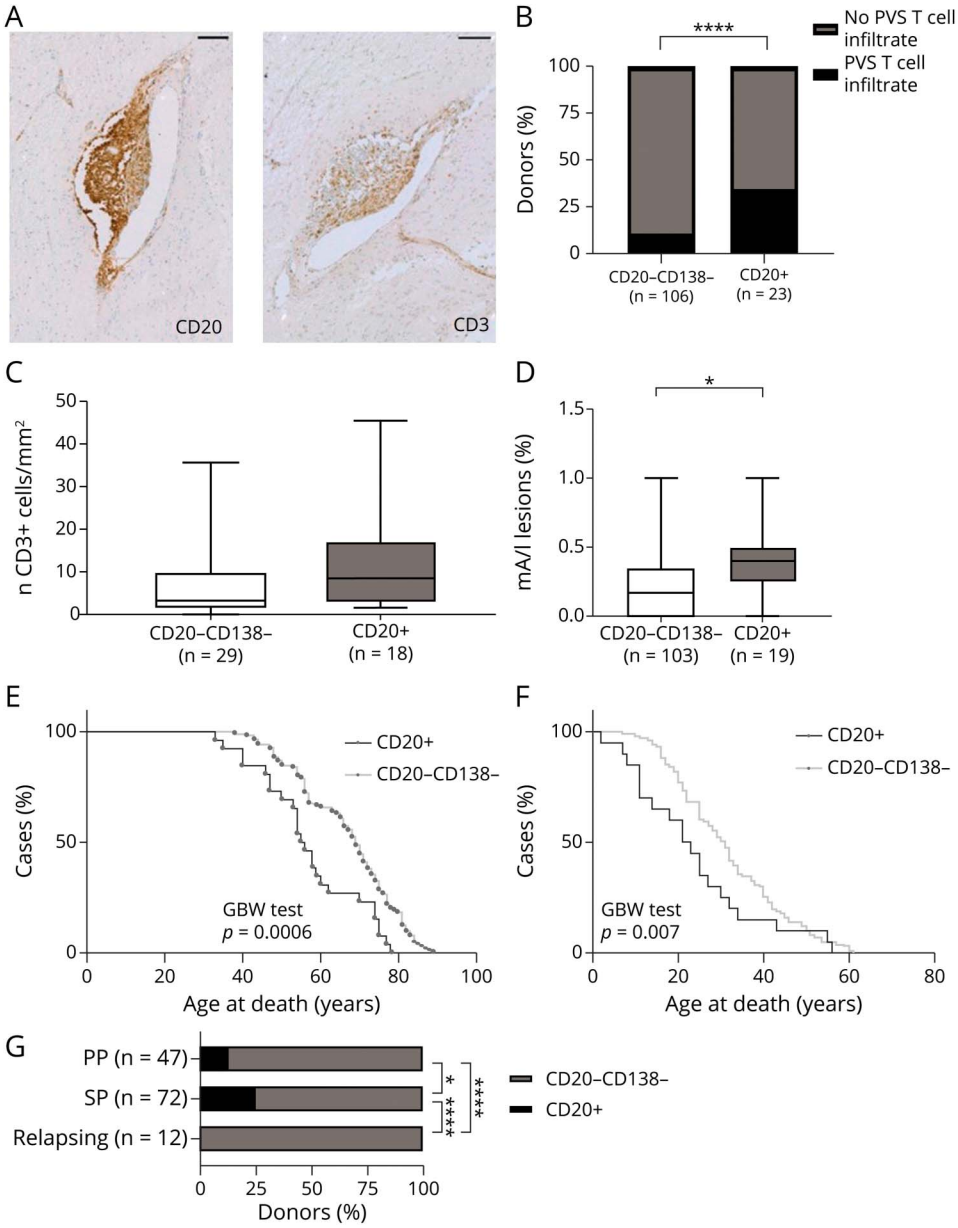
Because our data suggest an association between the presence of B cells in meninges and MO/subcortical WM, as well as an association with a more severe pathological and clinical profile, we explored its relevance for intrathecal B-cell activation. Because an increased intrathecal IgG production and OCB presence are highly correlating biomarkers of MS,[29] and the presence of OCB's is associated with adverse outcomes,[6,30] we explored whether these CSF biomarkers were associated with B-cell and CD138$^+$ 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$^+$ plasma cells in both MO and subcortical WM lesions. One MS case with B cells and CD138$^+$ 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$^+$ 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$^+$ 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$^+$ 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.[14] 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

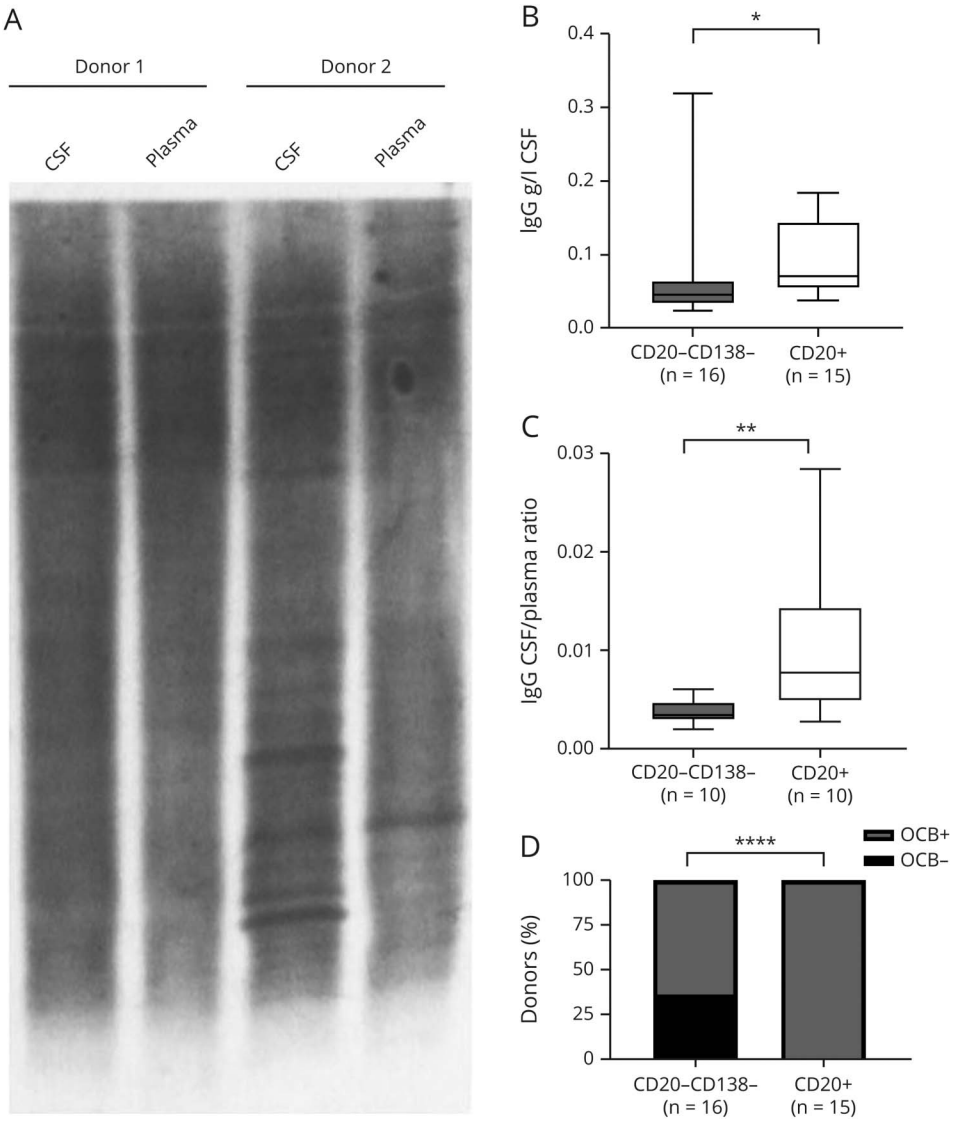
In MS autopsy cases selected for absence of B cells and CD138$^+$ plasma cells, the prevalence of OCBs was lower than the expected 90% OCB positivity of patients with MS at diagnosis.[31] This difference could be explained by selection of MS donors with an extreme profile of genetic or environmental factors,[16] 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$^+$ T cells, (C) a lower number of CD3$^+$ T cells in MS lesions, (D) a lower percentage of mixed active/inactive (mA/I) 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



(A) Example of OCB patterns in paired CSF and plasma for a donor without (donor 1) and a donor with (donor 2) CSF-unique OCBs. MS cases with limited presence of B cells showed (B) a lower concentration of IgG in postmortem CSF and (C) a lower CSF/plasma IgG ratio, suggesting a lower intrathecal IgG production, and (D) more often lacked CSF OCBs. *$p < 0.05$, **$p < 0.01$, and ****$p < 0.0001$. IgG = immunoglobulin G; OCB = oligoclonal band.

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 ± 8.5 (mean ± 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 $CD138^+$ 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 $CD138^+$ 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.[32–35] 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_{RM} cells are associated with the ongoing inflammatory lesion activity in WM lesions from advanced MS cases.[19,22,36] In MS lesions, these reactivated T_{RM} cells are often encountered in clusters in the perivascular space together with B cells,[17,37] suggesting that antigen presentation and reactivation of T_{RM} cells induced by B cells potentially occur at this location.[17,18,38] This illustrates that besides IgG production, B cells may have different functional roles in MS WM lesions.

We show that $CD138^+$ 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.[20,21] Of interest, $CD138^+$ plasma cells were most often present in inactive lesions compared with the other lesion subtypes. Prineas et al.[21] 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 $CD138^+$ 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 $CD20^+$ B cells but do not affect $CD138^+$ 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[39–41] and absence[39,42] of IgG deposits in MS lesions have been described. Also, the number of B cells in MS autopsy lesions is highly heterogenous 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.[4] 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.[16] 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.[19] 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 ± 8.5 (mean ± 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,[43] rituximab,[44] and alemtuzumab,[45] whereas treatment with natalizumab[46,47] and cladribine[48] was associated with reduced OCBs. Treatment with dimethyl fumarate has not been associated with lower CSF IgG production.[49] Although loss of CSF OCBs has been described in a cohort of interferon-beta– and glatiramer acetate–treated patients with MS,[50] 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.

Acknowledgment

The authors thank the MS brain donors for donating their brain and the NBB team for their excellent service, Jannette S. Langstraat for the measurements of IgG and OCBs, and Corbert G. van Eden for help with CD20 immunohistochemistry in the brainstem.

Study Funding

This research was funded by MS Research (grant MS 14-888) and the VriendenLoterij. J. Smolders was supported by the

Nationaal MS Fonds (OZ2018-003). T. Kuhlmann was supported by the German Research Foundation (CRCTR-128Z02).

Disclosure

N.L. Fransen reports no disclosures. B.A. de Jong received lecture and/or consultancy fees from Merck Serono, Biogen, Teva, Genzyme, and Novartis. K. Heß, T. Kuhlmann, M.C.J. Vincenten, and J. Hamann report no disclosures. I. Huitinga received lecture and/or consultancy fees from Biogen and Novartis. J. Smolders received lecture and/or consultancy fees from Biogen, Merck, Novartis, and Sanofi-Genzyme. Go to Neurology.org/NN for full disclosures.

Publication History

Received by *Neurology: Neuroimmunology & Neuroinflammation* July 4, 2020. Accepted in final form November 18, 2020.

Appendix Authors

Name	Location	Contribution
Nina L. Fransen, MD	Netherlands Institute for Neuroscience, Amsterdam, The Netherlands	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data
Brigit A. de Jong, MD, PhD	Amsterdam University Medical Centers, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; and analysis or interpretation of data
Katharina Heß, MD	University Hospital Münster, Münster, Germany	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; and analysis or interpretation of data
Tanja Kuhlmann, MD, PhD	University Hospital Münster, Münster, Germany	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; and analysis or interpretation of data
Maria C.J. Vincenten, MSc	Netherlands Institute for Neuroscience, Amsterdam, The Netherlands	Major role in the acquisition of data
Jörg Hamann, PhD	Netherlands Institute for Neuroscience, Amsterdam, The Netherlands; Amsterdam University Medical Centers, University of Amsterdam, Amsterdam, The Netherlands	Drafting/revision of the manuscript for content, including medical writing for content, and analysis or interpretation of data
Inge Huitinga, PhD	Netherlands Institute for Neuroscience, Amsterdam, The Netherlands; Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, The Netherlands	Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; and analysis or interpretation of data
Joost Smolders, MD, PhD	Netherlands Institute for Neuroscience, Amsterdam, The Netherlands; Erasmus Medical Center, Rotterdam, The Netherlands.	Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; and analysis or interpretation of data

References

1. Lublin FD, Reingold SC, Cohen JA, et al. Defining the clinical course of multiple sclerosis: the 2013 revisions. Neurology 2014:278–286.
2. Harrison DM, Li X, Liu H, et al. Lesion heterogeneity on high-field susceptibility MRI Is associated with multiple sclerosis severity. AJNR Am J Neuroradiol 2016;37: 1447–1453.
3. Hegen H, Auer M, Deisenhammer F. Predictors of response to multiple sclerosis therapeutics in individual patients. Drugs 2016;76:1421–1445.
4. Annunziata P, Giorgio A, De Santi L, et al. Absence of cerebrospinal fluid oligoclonal bands is associated with delayed disability progression in relapsing-remitting MS patients treated with interferon-beta. J Neurol Sci 2006;244:97–102.
5. Zeman AZJ, Kidd D, McLean BN, et al. A study of oligoclonal band negative multiple sclerosis. J Neurol Neurosurg Psychiatry 1996;60:27–30.
6. Tintore M, Rovira À, Río J, et al. Defining high, medium and low impact prognostic factors for developing multiple sclerosis. Brain 2015;138:1863–1874.
7. Kuhle J, Disanto G, Dobson R, et al. Conversion from clinically isolated syndrome to multiple sclerosis: a large multicentre study. Mult Scler 2015;21:1013–1024.
8. Lucchinetti CF, Popescu BF, Bunyan RF, et al Inflammatory cortical demyelination in early multiple sclerosis. N Engl J Med 2011;365:2188–2197.
9. Kutzelnigg A, Lucchinetti CF, Stadelmann C, et al Cortical demyelination and diffuse white matter injury in multiple sclerosis. Brain 2005;128:2705–2712.
10. Magliozzi R, Howell O, Vora A, et al. Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. Brain 2007;130:1089–1104.
11. Choi SR, Howell OW, Carassiti D, et al. Meningeal inflammation plays a role in the pathology of primary progressive multiple sclerosis. Brain 2012;135:2925–2937.
12. Howell OW, Reeves CA, Nicholas R, et al. Meningeal inflammation is widespread and linked to cortical pathology in multiple sclerosis. Brain 2011;134:2755–2771.
13. Serafini B, Rosicarelli B, Magliozzi R, Stigliano E, Aloisi F. Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis. Brain Pathol 2004;14:164–174.
14. Reali C, Magliozzi R, Roncaroli F, Nicholas R, Howell OW, Reynolds R. B cell rich meningeal inflammation associates with increased spinal cord pathology in multiple sclerosis.Brain Pathol 2020;30:779–793.
15. Luchetti S, Fransen NL, van Eden CG, Ramaglia V, Mason M, Huitinga I. Progressive multiple sclerosis patients show substantial lesion activity that correlates with clinical disease severity and sex: a retrospective autopsy cohort analysis. Acta Neuropathol 2018;135:511.
16. Fransen NL, Crusius JBA, Smolders J, et al. Post-mortem multiple sclerosis lesion pathology is influenced by single nucleotide polymorphisms. Brain Pathol 2020:30: 106–119.
17. Fransen NL, Hsiao C-C, van der Poel M, et al. Tissue-resident memory T cells invade the brain parenchyma in multiple sclerosis white matter lesions. Brain 2020:143; 1714–1730.
18. Smolders J, Heutinck KM, Fransen NL, et al. Tissue-resident memory T cells populate the human brain. Nat Commun 2018;9:4593.
19. Frischer JM, Bramow S, Dal-Bianco A, et al. The relation between inflammation and neurodegeneration in multiple sclerosis brains. Brain 2009;132:1175–1189.
20. Esiri MM. Immunoglobulin-containing cells in multiple-sclerosis plaques. Lancet 1977;2:478–480.
21. Prineas JW, Wright RG. Macrophages, lymphocytes, and plasma cells in the perivascular compartment in chronic multiple sclerosis. Lab Invest 1978;38:409–421.
22. Machado-Santos J, Saji E, Tröscher AR, et al. The compartmentalized inflammatory response in the multiple sclerosis brain is composed of tissue-resident CD8+ T lymphocytes and B cells. Brain 2018;141:2066–2082.
23. Waters PJ, Komorowski L, Woodhall M, et al. A multicenter comparison of MOG-IgG cell-based assays. Neurology 2019;92:E1250–E1255.

24. Waters P, Reindl M, Saiz A, et al Multicentre comparison of a diagnostic assay: aquaporin-4 antibodies in neuromyelitis optica. J Neurol Neurosurg Psychiatry 2016; 87:1005–1015.
25. Höftberger R, Guo Y, Flanagan EP, et al. The pathology of central nervous system inflammatory demyelinating disease accompanying myelin oligodendrocyte glycoprotein autoantibody. Acta Neuropathol 2020;139:875–892.
26. Chiaravalloti ND, DeLuca J. Cognitive impairment in multiple sclerosis. Lancet Neurol 2008;7:1139–1151.
27. Tellier J, Nutt SL. Standing out from the crowd: how to identify plasma cells. Eur J Immunol Wiley-vch Verlag 2017;47:1276–1279.
28. Freedman MS, Thompson EJ, Deisenhammer F, et al. Recommended standard of cerebrospinal fluid analysis in the diagnosis of multiple sclerosis: a consensus statement. Arch Neurol 2005;62:865–870.
29. Simonsen CS, Flemmen HØ, Lauritzen T, Berg-Hansen P, Moen SM, Celius EG. The diagnostic value of IgG index versus oligoclonal bands in cerebrospinal fluid of patients with multiple sclerosis. Mult Scler J 2020;6:205521731990129.
30. Ferreira D, Voevodskaya O, Imrell K, et al. Multiple sclerosis patients lacking oligoclonal bands in the cerebrospinal fluid have less global and regional brain atrophy. J Neuroimmunol 2014;274:149–154.
31. Calabrese M, Gasperini C, Tortorella C, et al. "Better explanations" in multiple sclerosis diagnostic workup: a 3-year longitudinal study. Neurology 2019;92:E2527–E2537.
32. Weber MS, Prod'homme T, Patarroyo JC, et al. B-cell activation influences T-cell polarization and outcome of anti-CD20 B-cell depletion in central nervous system autoimmunity. Ann Neurol 2010;68:369–383.
33. Brimnes MK, Hansen BE, Nielsen LK, Dziegiel MH, Nielsen CH. Uptake and presentation of myelin basic protein by normal human b cells. PLoS One 2014;9:e113388.
34. van Langelaar J, Rijvers L, Smolders J, van Luijn MM. B and T Cells driving multiple sclerosis: identity, mechanisms and potential triggers. Front Immunol 2020;11:760.
35. Molnarfi N, Schulze-Topphoff U, Weber MS, et al. MHC class II-dependent B cell APC function is required for induction of CNS autoimmunity independent of myelin-specific antibodies. J Exp Med 2013;210:2921–2937.
36. van Nierop GP, van Luijn MM, Michels SS, et al. Phenotypic and functional characterization of T cells in white matter lesions of multiple sclerosis patients. Acta Neuropathol 2017;134:383–401.
37. Revesz T, Kidd D, Thompson AJ, Barnard RO, McDonald WI. A comparison of the pathology of primary and secondary progressive multiple sclerosis. Brain 1994;117:759–765.
38. Corsiero E, Nerviani A, Bombardieri M, Pitzalis C. Ectopic lymphoid structures: powerhouse of autoimmunity. Front Immunol Front 2016;7:430.
39. Lucchinetti C, Brück W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. Ann Neurol 2000;47:707–717.
40. Breij ECW, Brink BP, Veerhuis R, et al. Homogeneity of active demyelinating lesions in established multiple sclerosis. Ann Neurol 2008;63:16–25.
41. Barnett MH, Parratt JD, Cho ES, Prineas JW. Immunoglobulins and complement in postmortem multiple sclerosis tissue. Ann Neurol 2009;65:32–46.
42. Prineas JW, Kwon EE, Cho ES, et al. Immunopathology of secondary-progressive multiple sclerosis. Ann Neurol 2001;50:646–657.
43. Kowarik MC, Pellkofer HL, Cepok S, et al. Differential effects of fingolimod (FTY720) on immune cells in the CSF and blood of patients with MS. Neurology 2011;76:1214–1221.
44. Cross AH, Stark JL, Lauber J, Ramsbottom MJ, Lyons JA. Rituximab reduces B cells and T cells in cerebrospinal fluid of multiple sclerosis patients. J Neuroimmunol 2006; 180:63–70.
45. Hill-Cawthorne GA, Button T, Tuohy O, et al. Long term lymphocyte reconstitution after alemtuzumab treatment of multiple sclerosis. J Neurol Neurosurg Psychiatry 2012;83:298–304.
46. Von Glehn F, Farias AS, De Oliveira ACP, et al. Disappearance of cerebrospinal fluid oligoclonal bands after natalizumab treatment of multiple sclerosis patients. Mult Scler 2012;18:1038–1041.
47. Mancuso R, Franciotta D, Rovaris M, et al. Effects of natalizumab on oligoclonal bands in the cerebrospinal fluid of multiple sclerosis patients: a longitudinal study. Mult Scler 2014;20:1900–1903.
48. Rejdak K, Stelmasiak Z, Grieb P. Cladribine induces long lasting oligoclonal bands disappearance in relapsing multiple sclerosis patients: 10-year observational study. Mult Scler Relat Disord 2019;27:117–120.
49. Høglund RA, Polak J, Vartdal F, Holmøy T, Lossius A. B-cell composition in the blood and cerebrospinal fluid of multiple sclerosis patients treated with dimethyl fumarate. Mult Scler Relat Disord 2018;26:90–95.
50. Mareš J, Herzig R, Urbánek K, et al. Relapsing-remitting multiple sclerosis and oligoclonal band pattern during disease modifying drug therapy relabující-remitující roztroušená skleróza a oligoklonální pruhy v průběhu léčby modifikující průběh choroby. Cesk Slov Neurol N 2007;103:674–677.