Anterior optic pathway pathology in CNS demyelinating diseases

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Background and rationale of the study

The anterior optic pathway is one of the preferential sites of disease involvement in CNS demyelinating diseases which all commonly feature optic neuritis as presenting symptom. Moreover, optical coherence tomography studies show that multiple sclerosis and neuromyelitis optica spectrum disorder also present a progressive neuroretinal thinning in absence of optic neuritis. We hypothesized that chronic inflammation in the anterior optic pathway leads to neurodegeneration, even in the absence of demyelination in MS and NMOSD. Thus we here characterise the extent of inflammation, demyelination, and neurodegeneration in the anterior optic pathway in MS, NMMOSD and ADEM.

Methods

We collected post-mortem tissue samples of optic nerves, chiasms, and tracts from 29 multiple sclerosis (age range 25-84 years, mean 59.48; 73 samples), 6 neuromyelitis optica spectrum disorders (age range 18-84 years, mean 56; 22 samples), 6 acute disseminated encephalomyelitis (age range 10-39 years, mean 25; 12 samples) and 5 non-neurological donors (age range 44-64 years, mean 55.2; 16 samples). Samples were immunolabelled for myelin - PLP, inflammation (microglial-macrophage – CD68/PGM1, T cells – CD4, B-cells – CD20, plasma-cells – CD138, complement – C9neo), acute axonal injury – B-APP, GFAP and AQP4.

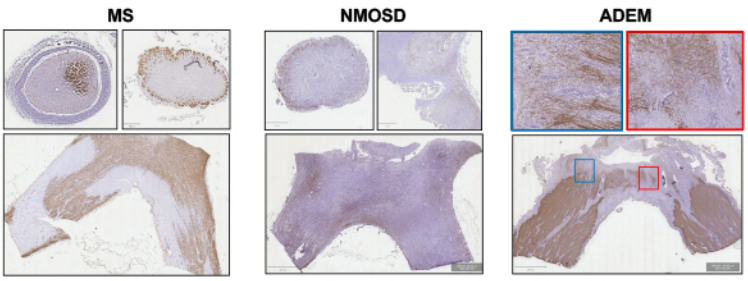
Method of analysis

Demyelination	*Inflammation*	*Axonal injury*
Area of demyelination	Macroglia-macrophage*	B-APP positive axons
Plaque activity	T-cell*, B-cells, plasmacells	
Presence of remyelination	Complement	

** Semiquantitative score (0 to 3) of perivascular, parenchymal inflammation; quantitative CD68+ pixel count; semiquantitative meningeal inflammation (0-2)*

Results

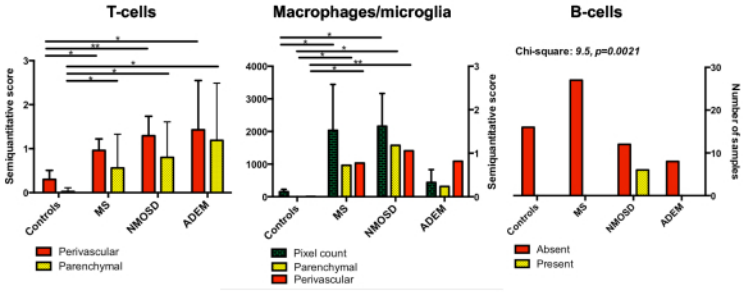
Plaque and plaque activity



MS	NMOSD	ADEM
24/29 cases (82.8%)	2/6 cases (33.3%)	2/6 cases (33.3%)
Proportional DM Area (52.6%)	*Proportional DM Area (52.6%)*	*Proportional DM Area (20%)*
ACTIVE: 18/24 cases (75%)	*ACTIVE: 2/2 cases*	*ACTIVE: 2/2 cases*
17% active, 58% mixed active	*50% active, 50% mixed active*	*100% active*

We found that demyelination affects 82.8% of multiple sclerosis patients and 75% of them present plaque activity at the moment of death, even in patients aged over 60 or with over 20 years of disease duration.

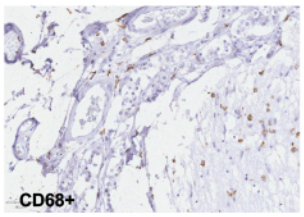
Inflammation in the Normal Appering White Matter



The presence of diffuse inflammatory changes along the NAWM is well-described in MS and we confirmed this evidence also in the pre-geniculate optic pathway. We found that cases aged more than 60 or with more than 20 years of disease duration tended to have higher inflammation in the NAWM compared with younger cases.
Interestingly we found similar findings considering the 4 NMOSD cases (18 samples) without evidence of demyelination along the pre-geniculate optic pathway. All these cases had no report of prior optic neuritis.

Meningeal Inflammation

T-cell and macrophage inflammation	MS	NMOSD	ADEM	Chi-square
Mild or severe combined	59%	**100%**	83.3%	p=0.006
Severe only	9.8%	31.3%	**50%**	p=0.044
Presence of CD20+	41%	**87.5%**	0%	p<0.001

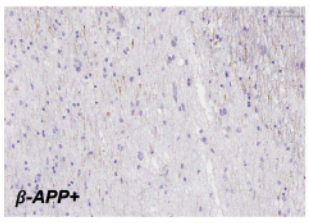


Meningeal samples of NMOSD cases invariably presented some degree of inflammation, whilst ADEM and MS displayed it in 83.3% and 59% of samples, respectively. The presence of meningeal inflammation was associated with plaque activity and the extent of microglial-macrophage inflammation in the NAWM.
B-cells were never detected in controls or ADEM, but were frequent in NMOSD and MS

Acute axonal injury

	*MS**	*NMOSD*	*ADEM*
Proportion cases with β-APP+ *(proportion samples with β-APP+)*	**41.4%** (27.4%)	**66.6%** *(54.5%)*	**50%** *(33.3%)*

*Not associated with disease duration or age



Acute axonal injury (B-APP positive axons) was frequently found in demyelinating CNS diseases, affected lesional and non-lesional tissue alike and its presence and extent was associated with inflammatory activity in the plaque, in the meningeal compartment and in the NAWM.

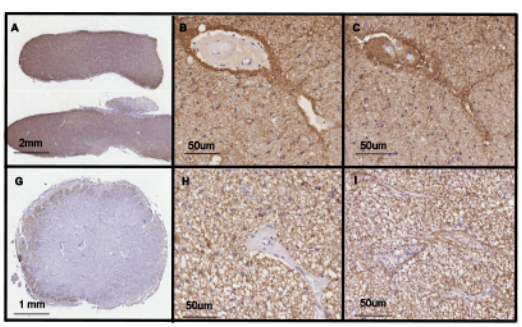
Antero-posterior gradient of disease involvement in MS

Optic nerves	Chiasms	Optic tracts
Extension of demyelinated area (%)		
65.2% *46.9 – 83.4%*	**45.2%** *18.9 – 71.4%*	**36.2%** *25.5 – 46.9%*
Wald Chi-square 8.22, p=0.016		
Remyelinated shadow plaques (presence)		
0% *0 out of 22 samples*	**15.4%** *2 out of 13 samples*	**27.3%** *3 out of 11 samples*
Linear-by-Linear Association 5.85, p = 0.025		
Inflammatory activity (presence of active plaques)		
69.6% *16 out of 23 samples*	**50%** *7 out of 14 samples*	**25%** *3 out of 12 samples*
Linear-by-Linear Association 6.2, p = 0.014		
Acute axonal injury (presence beta-APP positive axons)		
38.2% *13 out of 34 samples*	**31.6%** *6 out of 19 samples*	**5%** *1 out of 20 samples*
Linear-by-Linear Association 6.44, p = 0.012		

Pathological burden followed a clear antero-posterior gradient in MS samples with optic nerves showing a more extensive demyelination, a more intense inflammatory activity and acute axonal injury and a lower frequency of remyelinating plaques. NMOSD cases did not display a preferential pattern of involvement in our cohort.

GFAP and AQP4 expression in NMOSD

The NAWM of NMOSD cases shows a preserved GFAP and AQP4 patter compared with controls. While the plaque area shows the typical loss of the perivascular rosette pattern of both AQP4 and GFAP.
Therefore the inflammatory infiltrate in the NAWM was not associated with loss of AQP4 and GFAP.



Discussion and conclusions

Our findings suggest that chronic inflammation is frequently present in the plaque, in the meningeal compartment and in the NAWM. Chronic inflammation leads to continuous neurodegeneration both in multiple sclerosis and neuromyelitis optica, regardless of disease stage and prior optic neuritis history. The chronic inflammation and subsequent neurodegeneration occurring along the optic pathway may partly explain the neuroretinal changes observed in these diseases in-vivo, thus further enhance the relevance of monitoring the anterior optic pathway.