Supplemental Tables and Figures

Supplemental Table 1. Levels of biomarkers in acute NMOSD (discovery cohort) vs INDC and SC, discovery cohort

Marker type	NMOSD acute	INDC	P	SC	P‡
Number of samples	20	15		25	
nEla	114.8 [44.9, 670.8]	94.8 [49.8, 255.7]	1.000	13.2 [9.2, 17.6]	<0.001
MPO	7.9 [2.8, 73.7]	6.1 [2.5, 55.7]	0.610	1.0 [0.2, 2.2]	<0.001
NGAL	6410.5 [5173.0, 17147.5]	9479.0 [5188.0, 15685.0]	0.499	3751.0 [2221.0, 5111.0]	<0.001
MMP-8	8.7 [3.3, 127.3]	2.7 [2.3, 6.4]	0.043	2.2 [1.6, 2.7]	<0.001
MMP-9	270.2 [37.3, 2195.0]	9850.5 [832.3, 51865.4]	0.003	30.8 [20.3, 40.1]	0.006
TIMP-1	62660.0 [51160.0, 155750.0]	82970.0 [54600.0, 131450.0]	0.633	37490.0 [35530.0, 44650.0]	<0.001
GFAP	33958.8 [10730.5, 611223.2]	12125.3 [9313.4, 25050.4]	0.131	9818.0 [6725.0, 11551.7]	0.002
S100B	249.2 [186.0, 496.6]	301.1 [222.8, 402.4]	0.755	248.0 [175.4, 287.0]	0.230
NfL	1732.0 [1134.8, 3696.9]	1278.3 [448.4, 5334.6]	0.188	436.8 [316.3, 517.2]	<0.001
ICAM-1	3697.5 [2004.5, 6224.5]	4862.0 [3323.0, 9052.0]	0.131	2066.0 [1744.0, 2441.0]	0.004
VCAM-1	266900.0 [226175.0, 464200.0]	353000.0 [275600.0, 449000.0]	0.400	157400.0 [133700.0, 209000.0]	<0.001
CXCL13	11.4 [3.9, 47.9]	8.5 [2.5, 84.9]	0.877	0.6 [0.5, 0.9]	<0.001

Biomarker values are medians [IQR] in pg/ml. P values ≤0.05 are with green background.
‡P of comparison of acute NMOSD with SC.

Abbreviations: CXCL13: C-X-C motif chemokine 13; GFAP: glial fibrillary acidic protein; ICAM-1: intercellular adhesion molecule-1; INDC: inflammatory neurological disease control; MMP: matrix metalloproteinase; MPO: myeloperoxidase; nEla: neutrophil elastase; NGAL: neutrophil gelatinase-associated lipocalin; NfL: neurofilament light chain; NMOSD: neuromyelitis optica spectrum disorder; RRMS: relapsing-remitting multiple sclerosis; S100B: S100 calcium-binding protein B; SC: symptomatic control; TIMP-1: tissue inhibitor of metalloproteinase-1; VCAM-1: vascular cell adhesion molecule-1.

Supplemental Table 2. GAM levels in all NMOSD patients with and without corticosteroid pre-treatment

Corticosteroid pre-treatment (N)	No 23	Yes 44	P
Days between disease exacerbation and lumbar puncture; median [IQR]	13.0 [6.0, 43.5]	13.0 [7.0, 44.2]	0.982
nEla	199.7 [60.6, 409.9]	36.4 [13.0, 179.4]	0.006
MPO	8.2 [1.3, 385.3]	3.8 [0.3, 91.6]	0.214
NGAL	5095.0 [2423.0, 7837.0]	4480.0 [2180.0, 6760.9]	0.870
MMP-8	5.4 [3.0, 16.4]	3.6 [2.0, 18.4]	0.499
TIMP-1	52020.0 [21725.0, 63212.0]	45870.0 [30832.5, 63017.5]	0.891
S100B	225.3 [159.9, 606.2]	225.9 [182.7, 309.0]	0.900
ICAM-1	3531.0 [1305.5, 4488.0]	2312.5 [1826.0, 3772.5]	0.932
VCAM-1	230900.0 [159450.0, 31685.0]	212000.0 [158250.0, 305425.0]	0.716

Biomarker values are medians [IQR] in pg/ml. P values ≤0.05 are with green background.

Abbreviations: ICAM-1: intercellular adhesion molecule-1; MMP: matrix metalloproteinase; MPO: myeloperoxidase; nEla: neutrophil elastase; NGAL: neutrophil gelatinase-associated lipocalin; NMOSD: neuromyelitis optica spectrum disorder; S100B: S100 calcium-binding protein B; TIMP-1: tissue inhibitor of metalloproteinase-1; VCAM-1: vascular cell adhesion molecule-1.

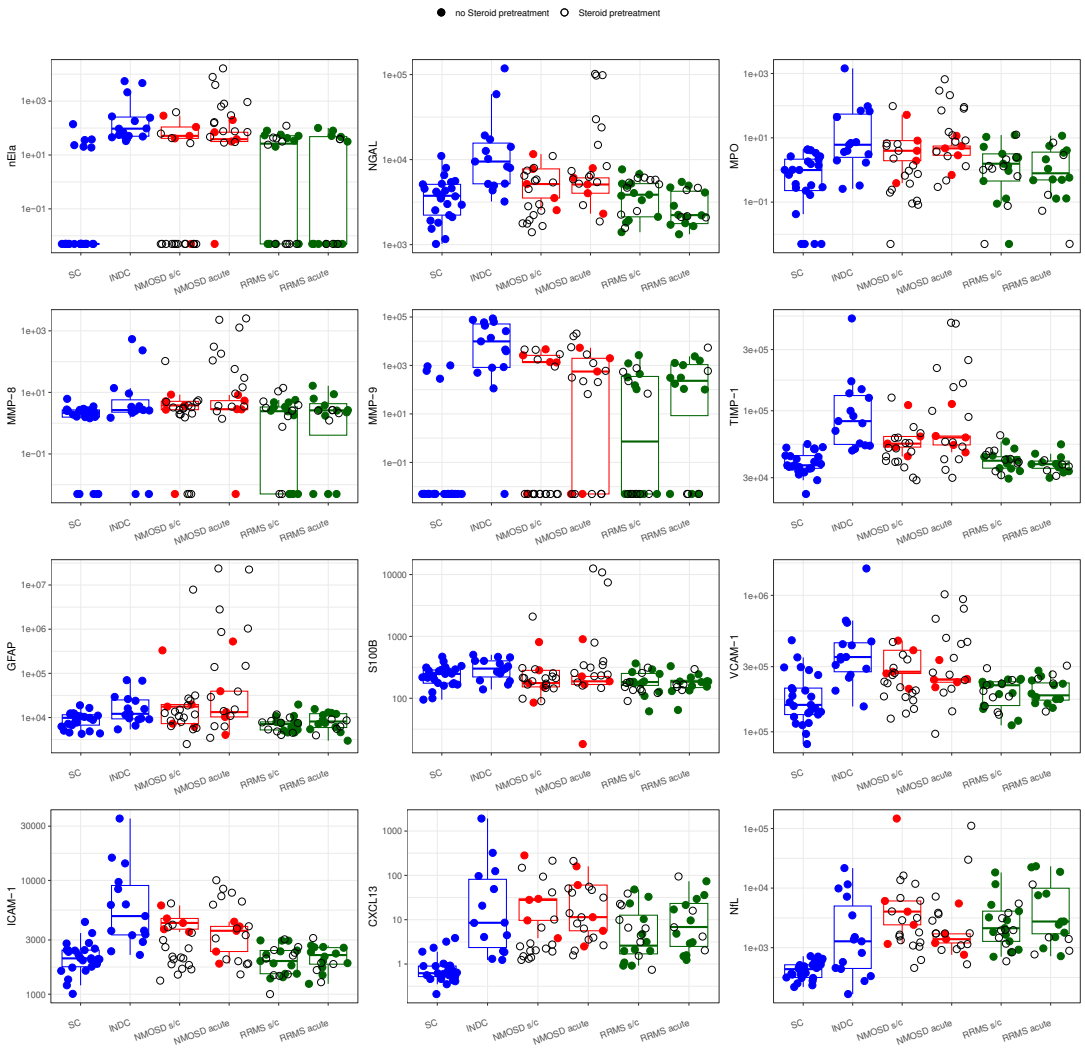
Supplemental Table 3. Association between EDSS and biomarkers in samples of NMOSD patients with and without corticosteroid pre-treatment

Marker	all NMOSD N=67		acute NMOSD N=41		s/c NMOSD N=26	
	Rho [CI]	p	Rho [CI]	p	Rho [CI]	p
nEla	0.38 [0.16-0.57]	0.001	0.24 [-0.11-0.48]	0.135	0.41 [0.03-0.69]	0.036
MPO	0.31 [0.08-0.52]	0.010	0.20 [-0.1-0.48]	0.213	0.21 [-0.19-0.56]	0.293
NGAL	0.46 [0.24-0.63]	<0.001	0.50 [0.22-0.70]	<0.001	0.27 [-0.13-0.60]	0.181
MMP-8	0.40 [0.18-0.59]	<0.001	0.40 [0.11-0.63]	0.009	0.27 [-0.13-0.60]	0.178
TIMP-1	0.35 [0.12-0.54]	0.004	0.39 [0.10-0.63]	0.011	0.28 [-0.12-0.60]	0.168
ICAM-1	0.39 [0.16-0.57]	0.002	0.47 [0.19-0.68]	0.002	0.18 [-0.22-0.53]	0.375
VCAM-1	0.38 [0.15-0.57]	0.001	0.46 [0.17-0.67]	0.003	0.14 [-0.26-0.50]	0.501

P values ≤0.05 are with green background.

Abbreviations: CXCL13: C-X-C motif chemokine 13; GFAP: glial fibrillary acidic protein; ICAM-1: intercellular adhesion molecule-1; MMP: matrix metalloproteinase; MPO: myeloperoxidase; nEla: neutrophil elastase; NGAL: neutrophil gelatinase-associated lipocalin; NfL: neurofilament light chain; NMOSD: neuromyelitis optica spectrum disorder; RRMS: relapsing-remitting multiple sclerosis; TIMP-1: tissue inhibitor of metalloproteinase-1; S100B: S100 calcium-binding protein B; VCAM-1: vascular cell adhesion molecule-1.

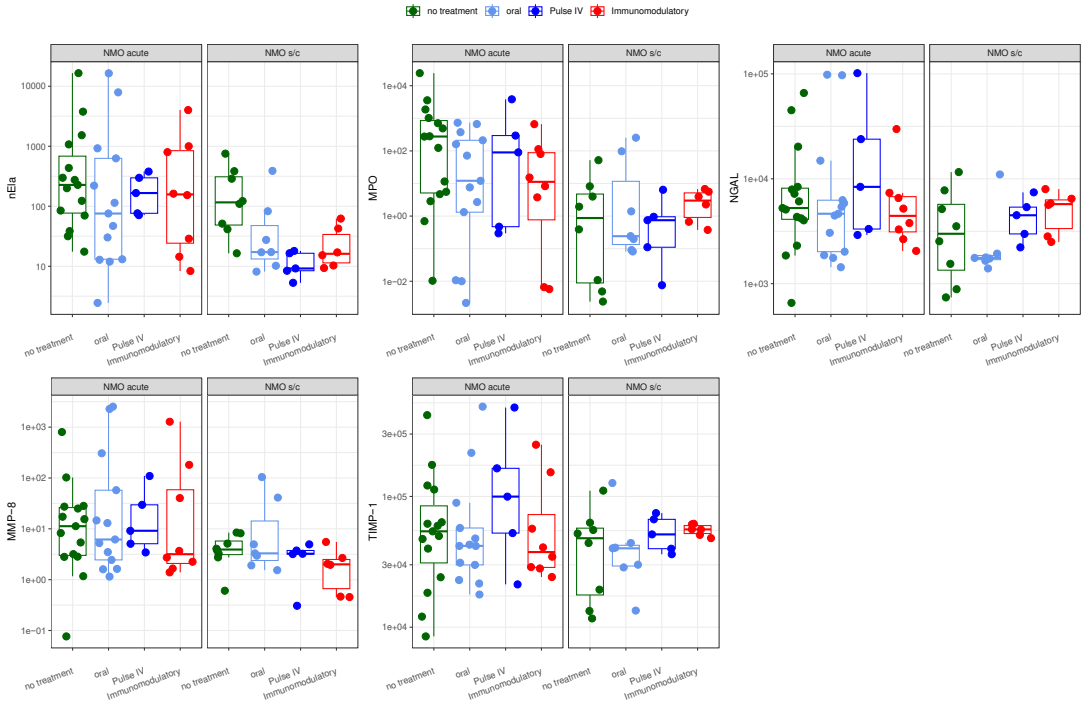
Supplemental Figure 1A. Biomarker levels in NMOSD, RRMS, SC and INDCs in discovery cohort



Boxplot of biomarker levels on log-scale (pg/ml) by patient population. Patients with corticosteroid pre-treatment (○) are not used for the boxplot calculations. Note that many patients under corticosteroid treatment in acute NMOSD have values above the 3rd quartile/75th percentile.

Abbreviations: CXCL13: C-X-C motif chemokine 13; GFAP: glial fibrillary acidic protein; ICAM-1: intercellular adhesion molecule-1; INDC: inflammatory neurological disease control; MMP: matrix metalloproteinase; MPO: myeloperoxidase; nEla: neutrophil elastase; NfL: neurofilament light chain; NGAL: neutrophil gelatinase-associated lipocalin; NMOSD: neuromyelitis optica spectrum disorder; RRMS: relapsing-remitting multiple sclerosis; TIMP-1: tissue inhibitor of metalloproteinase-1; S100B: S100 calcium-binding protein B; s/c: subacute/chronic; SC: symptomatic control; VCAM-1: vascular cell adhesion molecule-1.

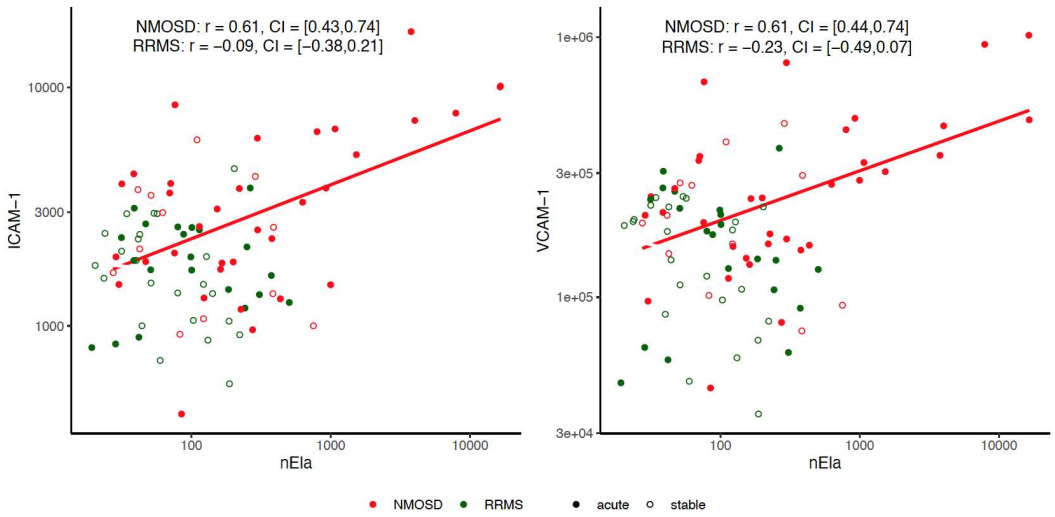
Supplemental Figure 1B: Effect of immunomodulatory therapy prior to lumbar puncture on marker levels in NMOSD patients



Boxplot of biomarker levels on log-scale (pg/ml) in acute and s/c NMOSD patients, subcategorised for 'no treatment' (●), 'oral steroids only' (●), 'pulse iv steroids' (●), and 'steroids + immunomodulatory therapy' (●).

Abbreviations: MMP: matrix metalloproteinase; MPO: myeloperoxidase; nEla: neutrophil elastase; NGAL: neutrophil gelatinase-associated lipocalin; NMOSD: neuromyelitis optica spectrum disorder; TIMP-1: tissue inhibitor of metalloproteinase-1.

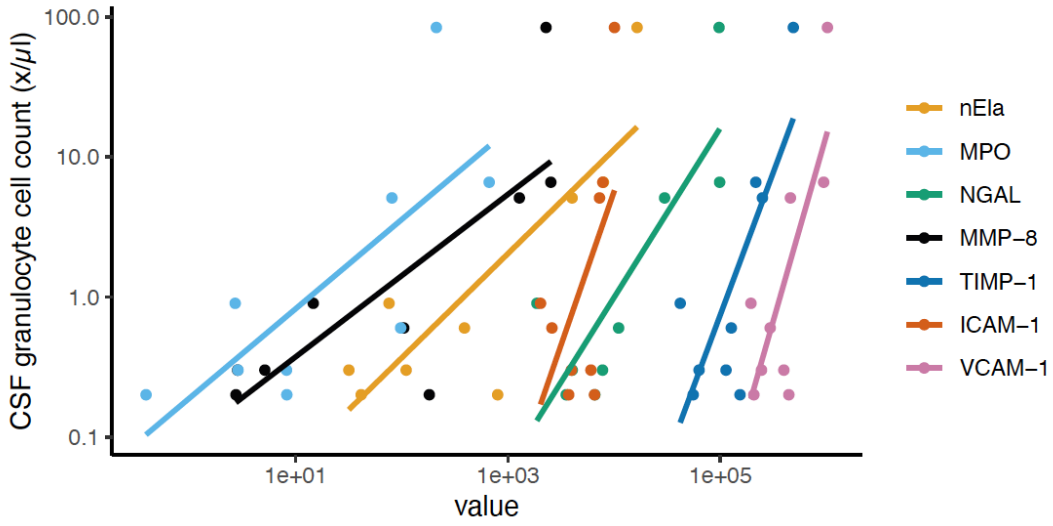
Supplemental Figure 2. Correlation of levels of elastase with ICAM-1 and VCAM-1 in pooled cohorts of NMOSD and RRMS patients.



CSF samples from patients (acute (●) and s/c (○) phase, without and with corticosteroid pretreatment) with quantifiable levels of nEla were correlated with levels of ICAM-1 and VCAM-1, respectively. Biomarker values are medians [IQR] in pg/ml. NMOSD: (red), RRMS: (green). Regression line is derived from samples of NMOSD patients with and without corticosteroid pretreatment. The exclusion of NMOSD patients with iv corticosteroid pre-treatment produced virtually identical regression line characteristics: ICAM-1: r=0. 65, CI =[0.47, 0.77]; VCAM-1: r=0.72, CI =[0.57, 0.82]. Regression line characteristics from NMOSD patients with iv or oral corticosteroid pre-treatment excluded were: ICAM-1: r=0. 56, CI =[0.30, 0.75]; VCAM-1: r=0.46, CI =[0.16, 0.68].

Abbreviations: CI: confidence interval; ICAM-1: intercellular adhesion molecule-1; nEla: neutrophil elastase; NMOSD: neuromyelitis optica spectrum disorder; RRMS: relapsing-remitting multiple sclerosis; VCAM-1: vascular cell adhesion molecule-1.

Supplemental Figure 3. Correlation of CSF granulocyte cell count ≥1 with biomarkers in discovery set NMOSD patients with and without corticosteroid pre-treatment



Biomarker values are in pg/ml on the x-axis. There was a strong (Rho 0.60-0.79) correlation of cell-specific GAM levels in those nine NMOSD patients with a CSF granulocyte cell count ≥1 (Rho, p-value: nEla =0.70, 0.037; MPO: 0.70, 0.037; MMP-8: 0.76, 0.018; NGAL: 0.69, 0.040), for TIMP-1 (0.60, 0.090) set significance levels were not reached.
In addition, strong correlation of CSF granulocyte cell count was present for S100B (0.77, 0.014) and GFAP (0.68, 0.044), and a moderate, but not significant correlation (Rho 0.40-0.59) for VCAM-1 (0.59, 0.096) and for ICAM-1 (0.50, 0.166). NfL and CXCL13 showed no correlation with the CSF granulocyte cell count.

Abbreviations: CXCL13: C-X-C motif chemokine 13; GFAP: glial fibrillary acidic protein; ICAM-1: intercellular adhesion molecule-1; MMP: matrix metalloproteinase; MPO: myeloperoxidase; nEla: neutrophil elastase; NfL: neurofilament light chain; NGAL: neutrophil gelatinase-associated lipocalin; NMOSD: neuromyelitis optica spectrum disorder; TIMP-1: tissue inhibitor of metalloproteinase-1; S100B: S100 calcium-binding protein B; VCAM-1: vascular cell adhesion molecule-1.