Supplemental material

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Granulocyte markers identify acute NMOSD

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.

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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.

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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.

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Supplemental Figure 1A. Biomarker levels in NMOSD, RRMS, SC and INDCs in discovery cohort
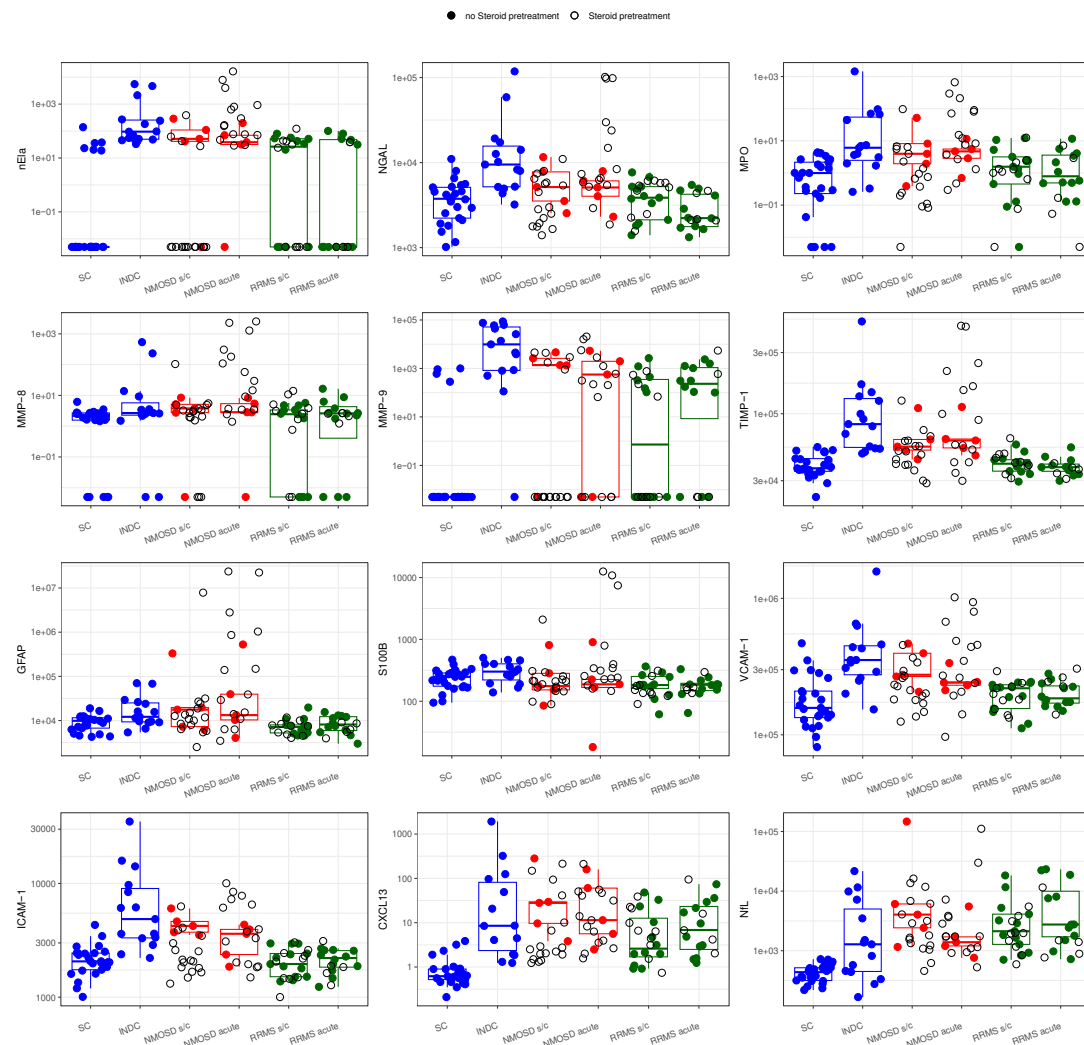
Boxplot of biomarker levels on log-scale (pg/ml) by patient population. Patients with corticosteroid pre-treatment (O) are not used for the boxplot calculations. Note that many patients under corticosteroid treatment in acute NMOSD have values above the 3rd quartile/75th percentile.

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.

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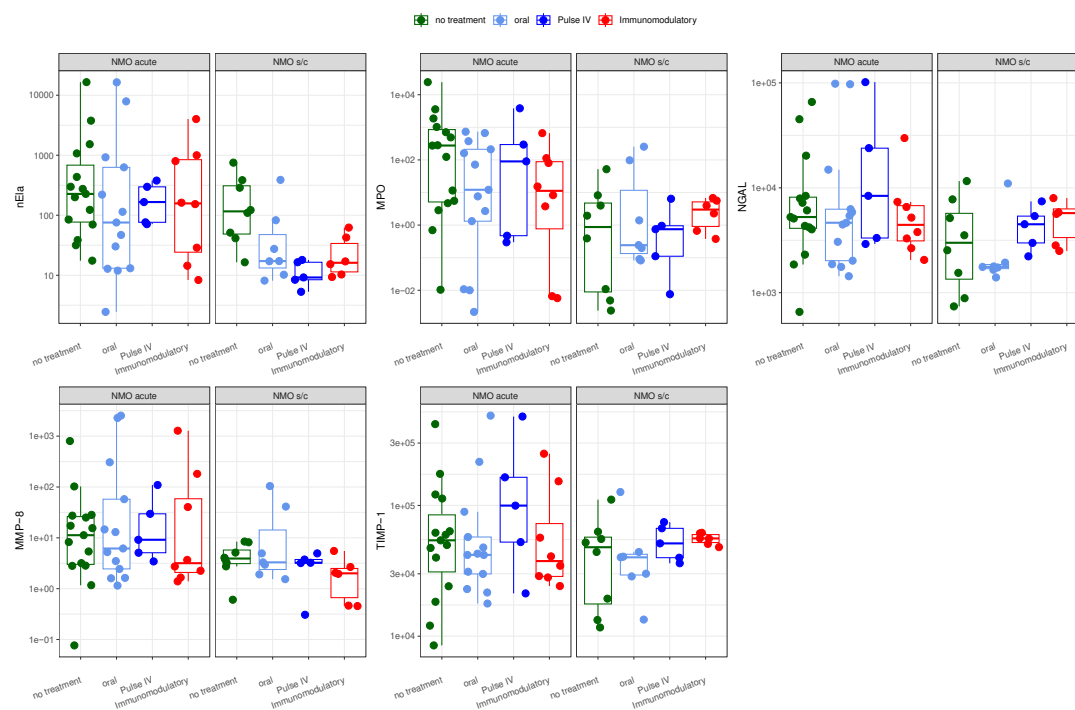

Boxplot of biomarker levels on log-scale (pg/ml) in acute and s/c NMOSD patients, subcategorised for 'no treatment' (●), 'oral steroids only' (●), 'pulse iv steroids' (●), and 'steroids + immunomodulatory therapy' (●).

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.

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Supplemental Figure 2. Correlation of levels of elastase with ICAM-1 and VCAM-1 in pooled cohorts of NMOSD and RRMS patients.
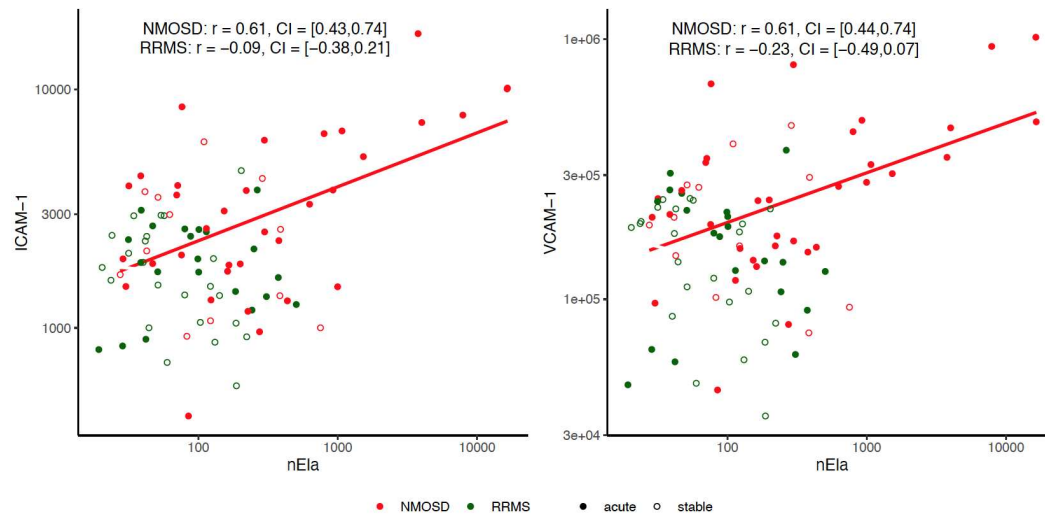
CSF samples from patients (acute (●) and s/c (○) phase, without and with corticosteroid pretreatment) with quantifiable levels of nEla were correlated with levels of ICAM-1 and VCAM-1, respectively. Biomarker values are medians [IQR] in pg/ml. NMOSD: (red), RRMS: (green). Regression line is derived from 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.

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Supplemental Figure 3. Correlation of CSF granulocyte cell count ≥1 with biomarkers in discovery set NMOSD patients with and without corticosteroid pre-treatment
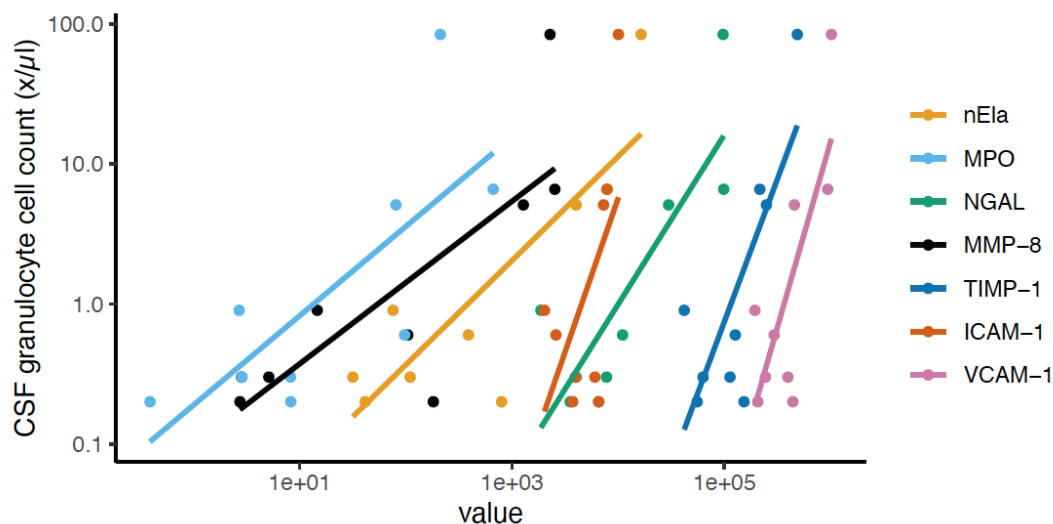
Biomarker values are in pg/ml on the x-axis. There was a strong (Rho 0.60-0.79) correlation of cell-specific GAM levels in those nine NMOSD patients with a CSF granulocyte cell count ≥1 (Rho, p-value: nEla =0.70, 0.037; MPO: 0.70, 0.037; MMP-8: 0.76, 0.018; NGAL: 0.69, 0.040), for TIMP-1 (0.60, 0.090) 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.